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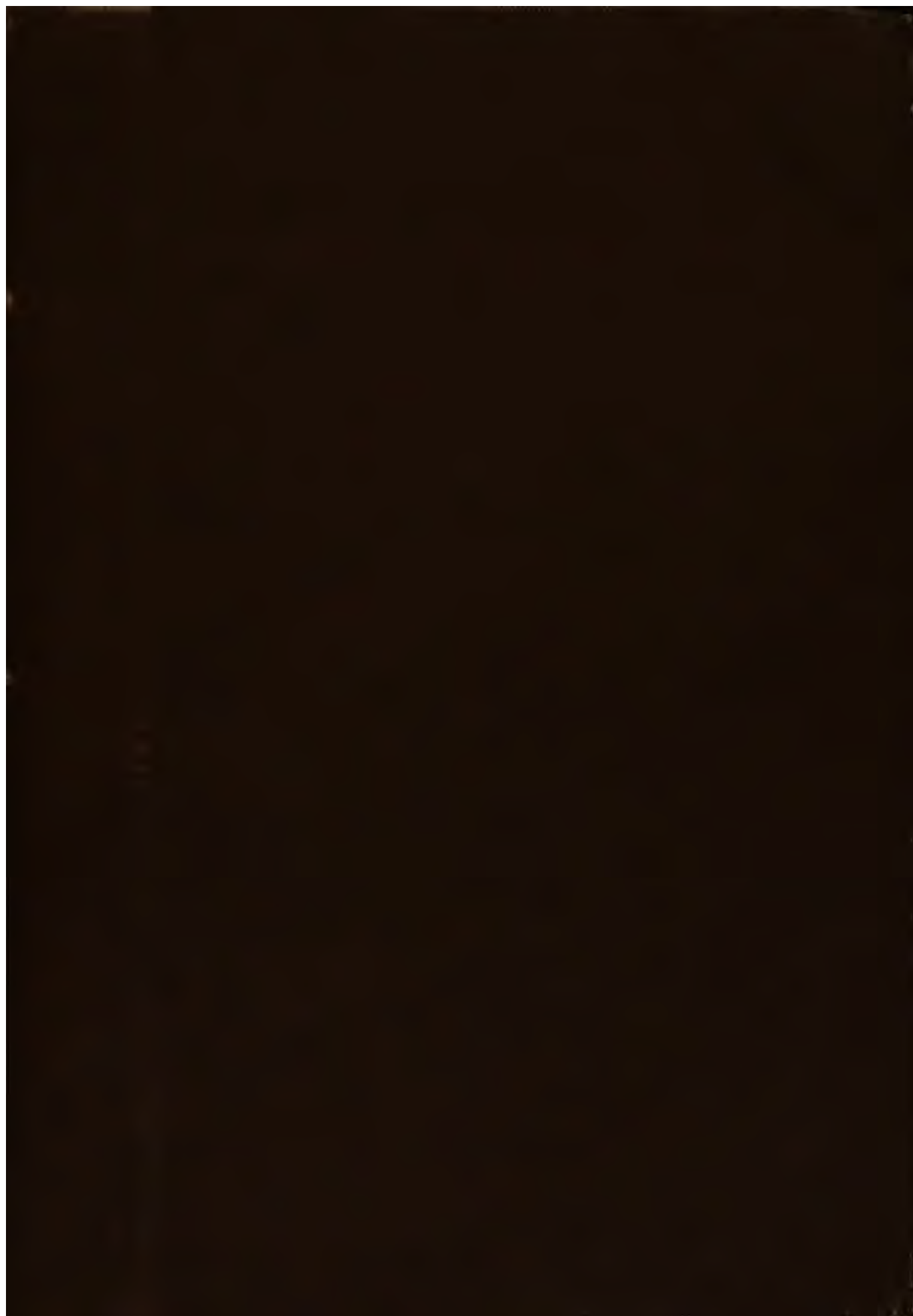
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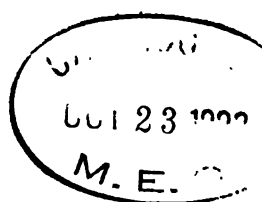
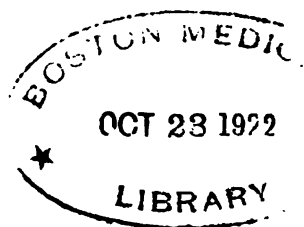
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CONTENTS

NUMBER 1, JANUARY, 1921

Effect of a Reduction of Lymphocytes on the Growth Rate of Transplanted Spontaneous Tumors in Mice. Frederick Prime.....	1
A Comparison of the Growth of Mice which Ultimately Develop Carcinoma with the Growth of Mice which Do Not Develop Carcinoma. T. Brailsford Robertson and L. A. Ray.....	7
Effect of Blood from Immune Animals upon Transplantable Tumors. Isidor Kross.....	25
Inoculation of Sarcomatous Tumors into Negro Fowls, with Special Reference to the Significance of Chromatophores. Yutaka Kon and Tamotsu Fujii.	31
Influence of the Lymphocyte on the Peritoneal Implantation of Sarcoma in Mice. Ellis Kellert.....	41
Primary Spontaneous Squamous Cell Carcinomas in Mice. Studies on the Incidence and Inheritability of Spontaneous Tumors in Mice. Fifteenth Communication. Maud Slye, Harriet F. Holmes and H. Gideon Wells...	57
Proceedings of the American Association for Cancer Research. Thirteenth Annual Meeting.....	87

NUMBER 2, APRIL, 1921

A Study of a Lipomyxosarcoma with Comments upon the Origin of the Fat Cell. Victor C. Jacobson.....	109
Parabiosis and Tumor Growth. Isidor Kross.....	121
The Protein Content of the Whole Blood and Plasma in Cancer. Ruth C. Theis.....	127
Problems in Cancer Research. Montrose T. Burrows.....	131
The Influence of Heredity in Determining Tumor Metastases. Studies in the Incidence and Inheritability of Spontaneous Tumors in Mice. Sixteenth Report. Maud Slye.....	139
Proceedings of the American Association for Cancer Research. Fourteenth Annual Meeting.....	175

NUMBER 3, JULY, 1921

Further Investigations on the Origin of Tumors in Mice. VII. Tumor Age and Tumor Incidence. Leo Loeb.....	197
Further Investigations of Disturbances of Blood Sugar Equilibrium in Their Relation to Neoplasia. G. L. Rohdenburg, O. F. Krehbiel, and A. Bernhard.....	223
Pregnancy and Tumor Growth. Isidor Kross.....	245
Is Cancer Mortality Increasing? Wendell M. Strong.....	251

NUMBER 4, OCTOBER, 1921

Cancer and Parasite. Isidor Kross.....	257
Teratomas and Their Relation to Age. H. E. Himwich.....	261
An Atypical Adenoma of the Pancreas Originating in Islet Tissue. Harry Goldblatt.....	277
The Influence upon the Growth of Transplanted Flexner-Jobling Rat Carcinoma of Hydrogen Ions and of Various Salts in Different Concentrations. Kanematsu Sugiura, Helen Miller Noyes, and K. George Falk....	285
Primary Spontaneous Tumors in the Kidney and Adrenal of Mice. Studies on the Incidence and Inheritability of Spontaneous Tumors in Mice. Seventeenth Communication. Maud Slye, Harriet F. Holmes, and H. Gideon Wells.....	305
A Critical Investigation of the Freund-Kaminer Reaction. Louis Herly.....	337
Is Cancer a Biological Phenomenon? Some Heretic Thoughts on Cancer. F. G. Gade.....	357
The Relation of Muscular Activity to Carcinoma. A Preliminary Report. Ivar Sivertsen and A. W. Dahlstrom.....	365

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EFFECT OF A REDUCTION OF LYMPHOCYTES ON THE GROWTH RATE OF TRANSPLANTED SPONTANEOUS TUMORS IN MICE

FREDERICK PRIME

*From Columbia University, George Crocker Special Research Fund, F. C. Wood,
Director*

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Recently much interest has been centered upon the hypothesis that the variation in the number of the hemal lymphocytes may exert an important immunal function, diminishing or increasing the resistance of animals to tumor inoculations. Experimentally an increase or decrease in these cells is most easily induced by *x*-rays, and with the improved modern technique more accurate quantitative estimations of the rays are obtainable than was possible a few years ago. We owe to Murphy and Ellis the observation that small doses of *x*-ray repeated over consecutive days reduce the lymphocyte count, without seriously affecting the health of the animal (1). The experiments hitherto conducted on this phase of the cancer problem have been largely directed to the study of the effects of lymphocytic alterations upon transplantable tumors, where growth had already been well established. Sittenfield (2), using the Flexner-Jobling rat carcinoma, a transplantable tumor which has been observed through many generations, found that neither increase nor reduction of the lymphoid elements in the blood had any influence upon either resistance or susceptibility to tumor growth, an observation which he confirmed in a later series of experiments (3). What influence, if any, might be observed upon the growth of spontaneous tumors when transplanted into such *x*-rayed animals has not as yet been satisfactorily determined. Murphy and Morton (4) found that such tumors transplanted to *x*-rayed animals

"acted much as heteroplastic tissues, retrogressing as the lymphoid tissue regenerates," and they also assert that the resistance of animals to the implantation of their own spontaneous tumor is increased by stimulating the production of lymphocytes with *x*-ray (5). Chambers, Scott, and Russ (6) have also reported experiments on one primary mouse tumor only. They observed a lowering of an animal's resistance to implantation after a single large generalized dose of *x*-ray. Since, however, a large proportion of spontaneous tumors either do not grow when transplanted into other animals or give a very small percentage of takes, it seemed of practical importance to test the other aspect of the problem and to ascertain whether resistance to implantation can be decreased by a reduction of the lymphocytes. If this were possible, it would provide an easy method of propagating many tumors of value in cancer research, which otherwise would be lost owing to our inability to transplant them successfully.

The spontaneous tumors used were derived chiefly from a special strain of "Lathrop" animals, of which this laboratory had a large number. They were inoculated into mice from the same "Lathrop" strain, as such a closely related strain offers a more favorable soil than stock of another breed. Forty-eight to seventy-two animals in each case were used, twenty-four being set aside as controls, and twenty-four to forty-eight kept for *x*-ray treatment.

To prepare the animals for inoculation they were given seven consecutive *x*-ray treatments according to Murphy's directions for reducing the number of lymphocytes. At the end of this time a spontaneous tumor was removed from its host and a small piece, about 0.002 gram in weight, was inoculated into the right groin of both the untreated controls and the mice whose lymphocytes had been greatly reduced by *x*-ray. Before starting the *x*-ray treatment, as well as twenty-four hours after the last dose of *x*-ray had been given, and at weekly intervals thereafter for six weeks, the blood of the *x*-rayed mice as well as that of the controls was counted. The blood pictures all showed a marked fall in the lymphocyte count after the

administration of seven consecutive doses of *x*-ray, with a gradual return to around the normal point by the end of six weeks. The controls during the early part of the experiment all showed a marked spontaneous increase in the lymphocyte count.

The experiments lasted ten weeks or more, no animals being included in the last series of counts which had not survived for this period, and many of the series were observed over a period of twenty weeks. The results were quite different from those reported by Murphy, who used tumors which had been transplanted for many generations. He records that a reduction of the lymphocytes in the circulating blood was attended by an increase in the number of takes. In our experiments there was found to be practically no difference at all in the inoculation percentage between the untreated controls and those which had been given a reducing dosage of *x*-ray. The lymphocyte count in many of the controls was higher at the end than at the beginning of the experiment, and there should, therefore, according to the DaFano-Murphy theory, have been a smaller number of takes among them; but this was not the case.

Out of a series of 905 animals living at the end of five weeks which had been treated with *x*-ray, 72, or 7.9 per cent, showed tumors, and 92.1 per cent were negative; while out of 740 controls which were living, 6 per cent showed tumors, and 94 per cent were negative. At the end of ten weeks, out of 740 living animals which had been treated with *x*-ray, 4.3 per cent showed tumors, and 95.7 per cent showed no growths at all; and out of 615 controls, 3.7 per cent of the animals showed tumor growths, and 96.3 per cent were negative. A difference of 2 per cent in the number of takes at the end of five weeks between the animals whose lymphocytes had been reduced and the controls has no significance, and a difference of 0.6 per cent at the end of ten weeks shows how little evidence there is in favor of the theory that the lymphocyte plays any important part in the production of immunity in relation to transplanted cancer (see chart 1). If the DaFano-Murphy hypothesis that the lymphocyte plays an important rôle in the production of

immunity were correct, a reduction in the number of tumor takes should have occurred in these experiments; but, as has been shown, such was not the case.

That the x-ray treatment was sufficient to reduce materially the number of lymphocytes in the circulating blood was clearly demonstrated by the blood counts; and at the end of four weeks,

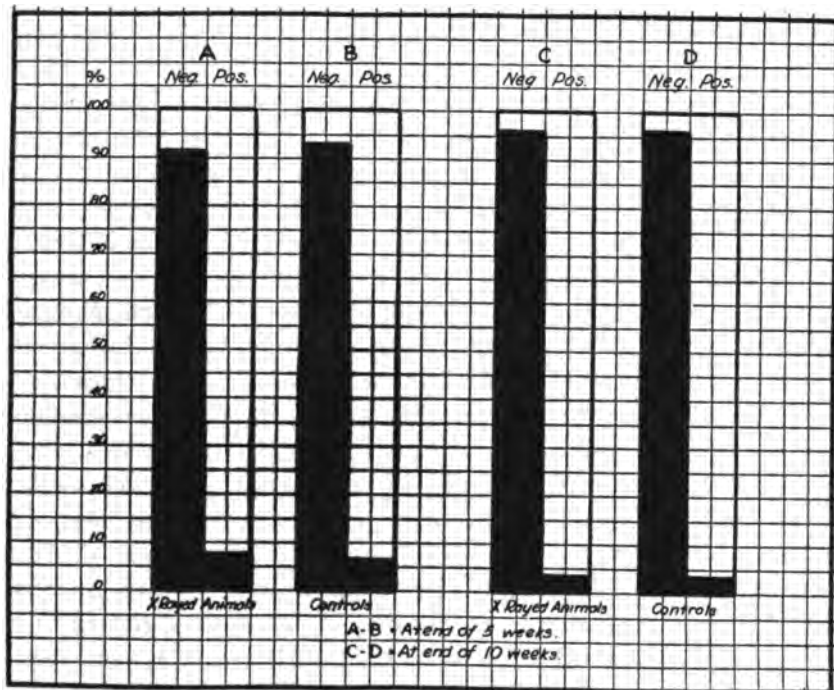


CHART 1

ample time for some evidence of tumor growth to be seen, the blood had not yet returned to normal. Whether this condition applies to spontaneous tumors alone, or to transplantable tumors in general, will have to be demonstrated for each tumor.

In the series reported, over forty spontaneous tumors were used and inoculations were made into 2100 mice.

CONCLUSIONS

Decreasing the circulating lymphocytes by small doses of x-ray does not render mice more susceptible to the inoculation

of a spontaneous tumor from another mouse of the same strain.

Mice with spontaneous lymphocytosis are not resistant to the implantation of a primary tumor from another mouse of the same strain.

REFERENCES

- (1) MURPHY, J. B., AND ELLIS, A. W. M.: Experiments on the rôle of lymphoid tissue in the resistance to experimental tuberculosis in mice. *J. Exper. M.*, **20**: 397, 1914.
- (2) SITTENFIELD, M. J.: The significance of the lymphocyte in immunity to cancer. *J. Cancer Res.*, **2**: 151, 1917.
- (3) SITTENFIELD, M. J.: Further studies on the importance of the lymphocyte in cancer immunity. *J. M. Res., N. S.* **33**: 463, 1918.
- (4) MURPHY, J. B., AND MORTON, J. J.: The lymphocyte in natural and induced resistance to transplanted cancer. *J. Exper. M.*, **22**: 204, 1915.
- (5) MURPHY, J. B., AND MORTON, J. J.: The effect of Roentgen-rays on the rate of growth of spontaneous tumors in mice. *J. Exper. M.*, **22**: 800, 1915.
- (6) CHAMBERS, H., SCOTT, G., AND RUSS, S.: On the action of x-rays upon the transplantation of a spontaneous carcinoma of the rat. *J. Path. and Bacteriol.*, **23**: 384, 1919-20.

A COMPARISON OF THE GROWTH OF MICE WHICH ULTIMATELY DEVELOP CARCINOMA WITH THE GROWTH OF MICE WHICH DO NOT DEVELOP CARCINOMA

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STATEMENT OF THE PROBLEM

Since cancer is a disease of growth, and its incidence in appreciable form is usually deferred until after the attainment of maturity, a very important factor in our ultimate understanding of the etiology of cancer must arise out of a knowledge of the degree to which the preceding development of the animal is affected by the ultimate aberration of growth. If the factors which determine the growth and incidence of carcinoma, for example, are of an essentially accidental character, such as local irritation *plus* infection, then the incidence of carcinoma in an individual will be decided in the main by factors not inherent in its habit of growth and metabolism, and the preceding normal development cannot be expected to foreshadow the impending pathological outcome. If, on the contrary, the incidence of carcinoma is determined by internal factors of growth-habit or metabolic peculiarities *plus* recurrent external factors, such as local irritation, then the underlying peculiarity of growth or metabolism may conceivably reveal itself in more or less marked departures from the average in the preceding normal development.

¹ The expenses of this research were in part defrayed by a grant from the special medical research fund of the University of Toronto.

A comparison throughout the duration of life of the growth of animals which ultimately develop spontaneous cancer and those which do not is, therefore, fundamental to our comprehension of the etiology of this disease. The fact that such a comparison has not hitherto been instituted is undoubtedly attributable to the experimental labor which is essential to obtain results of any worth, to the expenditure of time which is requisite, and to the difficulty of an experimental technique which must obviously be devised to exclude all chance causes of death, among which must be reckoned epidemic infections. It is obvious that if epidemic infections were from time to time to claim a substantial proportion of victims among the experimental animals, it would become impossible to identify the individuals which, had they survived the infection, would subsequently have developed carcinoma.

METHODS OF RESEARCH

For the past six years we have been engaged upon an extensive series of experiments of which the immediate object was the determination of the influence of certain glandular extracts or of normal food constituents in extraordinary excess upon the growth of otherwise normal white mice. The unusual constituent was added to an otherwise varied and abundant diet of barley, egg, biscuit, and lettuce. It was essential to our inquiry that epidemic infections should be excluded, and we were so far successful that mortality from these diseases, throughout the duration of their lives, did not amount to more than 4 per cent of the total deaths in the males, and 1 per cent in the females. The animals were weighed once a week from the fourth or fifth week after birth until the thirtieth week, and thereafter once a fortnight. Individual records of the growth of each mouse were kept, and its ultimate fate recorded. All lesions manifest to the naked eye in postmortem examination of the organ were noted, and sections of formaldehyde-hardened material were prepared and examined microscopically. We feel confident that nearly all cases of carcinoma and other new growths were detected and

identified except in a very small proportion of cases in which postmortem examination of the tissues was rendered impossible by delayed observation of death and consequent decomposition.

The details of our experimental technique and the growth and mortality statistics thus obtained have been fully described elsewhere (1).

By segregating, in each experimental group, the records of those animals which ultimately developed carcinoma, we have been able to compare the growth of those animals which developed carcinoma with the growth of those animals which did not, in eleven different experimental groups, involving a total of 324 animals, of which 105 developed spontaneous carcinoma. The primary growth was usually situated in the axilla or the groin, and metastases occurred in the lungs. In a small proportion of cases in the other groups and in every case in the group receiving tethelin throughout the duration of life (tethelin-fed males) the only new growths detectable by naked eye examination were situated in the lung; and these, in the tethelin-fed group, were very small, frequently but 1 to 4 mm. in diameter. In the more typical cases, involving a primary growth in the axilla or groin, death usually occurred within one month of the appearance of a manifest lump.

The diet of the "normal" animals consisted of crushed barley and water *ad libitum*, fresh leaves of lettuce twice a week, dry hard unsweetened biscuit ("pilot bread") once a week, and 5 cc. of mixed white and yolk of egg to every six animals on each of six days of the week. The egg was employed as the vehicle for the administration of the various dietary additions to the other groups; it was generally consumed within a few minutes of its introduction into the cage.

The pituitary-fed animals received one-half of an anterior lobe of an ox pituitary per six animals, emulsified in the daily allowance of egg. The lecithin, prepared from yolks of eggs, was administered in dosage corresponding to 83 mgm. per mouse per day. The dosage of cholesterol was 42 mgm. per mouse per day. The dosage of tethelin (a lipin prepared from the anterior lobe of the pituitary gland) was 4 mgm. per mouse per day. In one

group (tethelin-fed males) the administration of tethelin was continued throughout life. To one group of females ("discontinuous administration") this dose of tethelin was administered in three periods of one month each, namely from the end of the fourth week until the end of the eighth, from the end of the twenty-first until the end of the twenty-fifth, and from the end of the forty-second until the end of the forty-sixth week of life. To another group of females ("brief administration") the administration was continued only from the end of the fourth until the end of the twelfth week, and thus ceased before or at about the attainment of sexual maturity. Weighings, in this latter group, were discontinued between the thirtieth and the seventieth week because, mistakenly as events proved, no noteworthy effects of the administration upon normal growth subsequent to the thirtieth week were anticipated.

EXPERIMENTAL RESULTS

The incidence of carcinoma in the various experimental classes and the average duration of life of the animals in each class and of those which developed carcinoma are enumerated in table 1.² It will be observed that the percentage of incidence of carcinoma was substantially unaffected by the various dietaries employed, with the exception that the incidence was extraordinarily low (17 per cent) in the group of females which received the "brief administration" of tethelin. Traces of the same effect may be seen in the low incidence (25 per cent) in the group which received "discontinuous administration" of tethelin, the normal incidence in females being, for the strain employed, from 30 to 50 per cent. We shall see that of all the growth curves herein presented that for the "brief administration" tethelin group departed most widely from the type of growth curve generally displayed by animals which subsequently developed carcinoma. The high incidence of carcinoma in the pituitary-fed groups may possibly be fortuitous, or, again, may be attributable to the pituitary

² Owing to the cost of publication it has been necessary to omit many tables bearing in detail the data of the experiments.—*Editor*.

tissue, or, yet again, to the fact that the diet of these animals differed from that of all other classes in containing a proportion of raw meat.

The results of the comparison of the growth of the animals which ultimately developed carcinoma with the growth of the animals which did not are displayed graphically in Figures 1 to 11, in which the full lines represent the growth of the animals which did not, and the broken lines represent the growth of the

TABLE 1

Incidence of carcinoma in the various experimental classes and the duration of life of each

CLASS OF ANIMALS	ANIMALS WHICH DEVELOPED CARCINOMA	AVERAGE DURATION OF LIFE OF ALL ANIMALS	AVERAGE DURATION OF LIFE OF THE ANIMALS WHICH DEVELOPED CARCINOMA
Males			
	<i>per cent</i>	<i>days</i>	<i>days</i>
Normal.....	25	767	826
Pituitary.....	29	792	823
Lecithin.....	22	731	743
Cholesterol.....	26	764	848
Tethelin.....	35	866	958
Females			
Normal.....	37	719	721
Pituitary.....	51	704	689
Lecithin.....	32	731	743
Cholesterol.....	50	658	590
Tethelin (discontinuous administration).....	25	800	970
Tethelin (brief administration).....	17	695	677

animals which did develop carcinoma. The comparison is discontinued in each case at the date at which half of the carcinoma animals had died.

An examination of these curves reveals a striking uniformity of result. In each of the eleven different experimental groups the carcinomatous animals were superior in weight to the non-carcinomatous throughout a large proportion of their lives, and this occurred independently of whether, at the beginning of the weighings at four or five weeks of age, they were superior, equal,

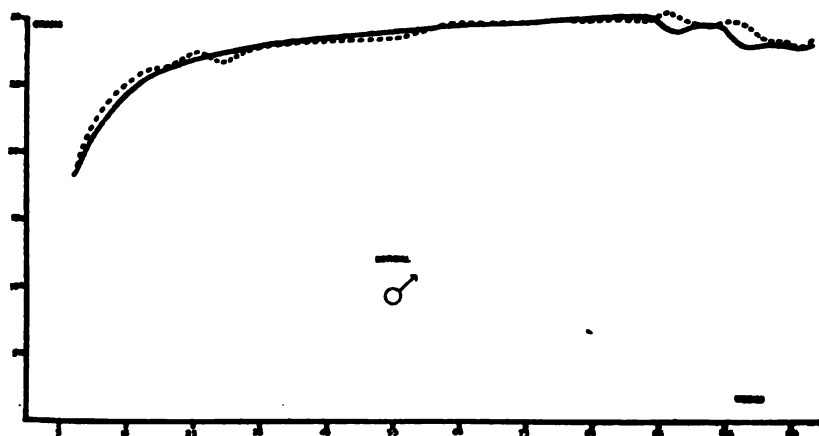


FIG. 1. COMPARISON OF THE GROWTH OF NORMAL MALE WHITE MICE WHICH ULTIMATELY DEVELOPED CARCINOMA (BROKEN LINE) WITH THAT OF NORMAL MALE WHITE MICE WHICH DID NOT DEVELOP CARCINOMA (FULL LINE)



FIG. 2. COMPARISON OF THE GROWTH OF NORMAL FEMALE WHITE MICE WHICH ULTIMATELY DEVELOPED CARCINOMA (BROKEN LINE) WITH THAT OF NORMAL FEMALE WHITE MICE WHICH DID NOT DEVELOP CARCINOMA (FULL LINE)

or inferior in weight to the non-carcinomatous animals. The superior growth occurred during the period of developing sexual maturity (adolescent growth) and was merely maintained or slightly diminished or even lost during later life. These facts are summarized in table 2.

It will be seen that in four out of the eleven groups the animals which developed carcinoma were initially inferior in weight to

TABLE 2

Relative weights at different ages of animals which developed carcinoma and those which did not. + indicates superiority of the animals which developed cancer; - indicates their inferiority in weight, and = indicates substantial equality of the two groups

CLASS OF ANIMALS	INITIAL WEIGHT (4 TO 5 WEEKS	10 TO 30 WEEKS	30 TO 70 WEEKS	70 WEEKS TO DEATH OF ONE HALF OF ANIMALS WHICH DEVELOPED CARCINOMA
Males.				
Normal.....	=	+	=	=
Pituitary.....	=	+	-	-
Lecithin.....	+	+	+	=
Cholesterol.....	+	+	+	+
Tethelin.....	=	+	+	+
Females				
Normal.....	=	+	+	=
Pituitary.....	-	+	+	+
Lecithin.....	-	=	+	+
Cholesterol.....	-	+	+	+
Tethelin (discontinuous adminis- tration).....	-	+	+	-
Tethelin (brief administration).....	+	+	+	+

the animals which did not subsequently develop carcinoma. Yet even in these cases superiority of weight was ultimately attained, and in three out of the four groups superiority of weight was attained before the thirtieth week. In the remaining group (lecithin-fed females) the initial inferiority was very great (1.65 grams, or 15 per cent of the mean weight) yet this group attained equality to the non-carcinomatous animals before the thirtieth week, and surpassed them thereafter.

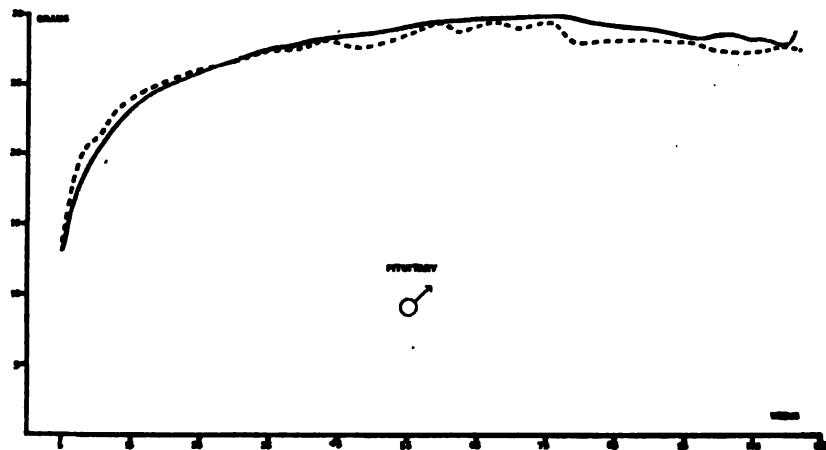


FIG. 3. COMPARISON OF THE GROWTH OF PITUITARY FED MALE WHITE MICE WHICH ULTIMATELY DEVELOPED CARCINOMA (BROKEN LINE) WITH THAT OF PITUITARY FED MALE WHITE MICE WHICH DID NOT DEVELOP CARCINOMA (FULL LINE)

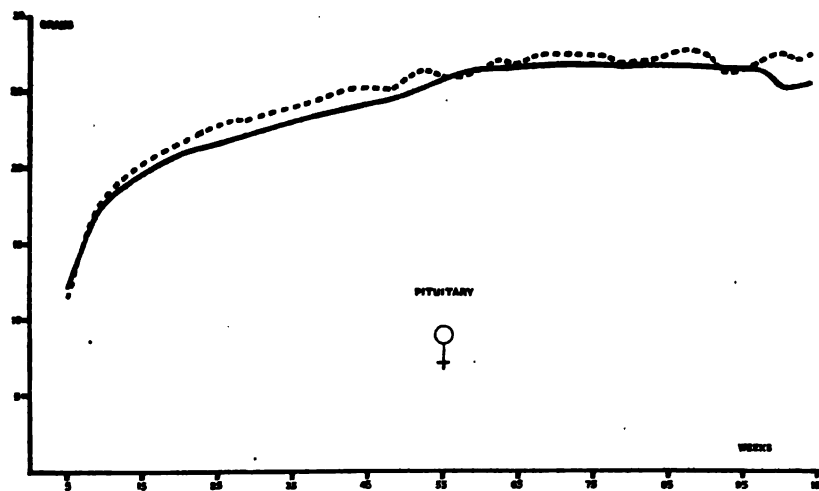


FIG. 4. COMPARISON OF THE GROWTH OF PITUITARY FED FEMALE WHITE MICE WHICH ULTIMATELY DEVELOPED CARCINOMA (BROKEN LINE) WITH THAT OF PITUITARY FED FEMALE WHITE MICE WHICH DID NOT DEVELOP CARCINOMA (FULL LINE)

INTERPRETATION OF THE RESULTS

We may infer that the animals which ultimately develop carcinoma foreshadow this outcome in their development during the earlier portions of the third or adolescent growth-cycle. They are distinguished from the animals which do not subsequently develop carcinoma by their exceptionally energetic growth during the period of adolescence. Prior to this period they do not display any uniform characteristics of weight, and subsequently to the thirtieth week the growth curve of the carci-

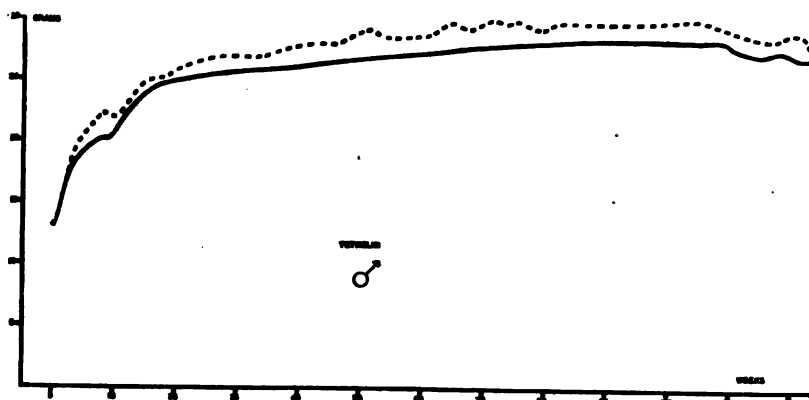


FIG. 5. COMPARISON OF THE GROWTH OF TETHELIN FED MALE WHITE MICE WHICH ULTIMATELY DEVELOPED CARCINOMA (BROKEN LINE) WITH THAT OF TETHELIN FED MALE WHITE MICE WHICH DID NOT DEVELOP CARCINOMA (FULL LINE)

nomatous animals usually remains parallel to the curve for the non-carcinomatous animals or approaches it, the only marked exception to this rule being afforded by the group of cholesterol-fed females in which the animals which subsequently developed carcinoma display a late accretion of weight between the forty-fifth and the sixty-fifth week.

The same data have afforded material for another significant comparison, namely, that of the growth of animals which survived beyond the average duration of life in any given experimental group with the growth of the animals which failed to attain the average duration of life (2). In this comparison

it was shown that the long-lived animals are distinguished by relatively energetic early growth, low variability, steadiness of weight, and absence of late accretions of weight. The short-lived animals, on the contrary, display relatively deficient early growth, high variability, instability of weight, and a marked tendency to acquire late accretions of weight.

The animals which ultimately develop carcinoma, therefore, display the general characteristics of the long-lived groups of animals. At first sight this is not surprising, for the average duration of life of the animals which develop carcinoma exceeds,

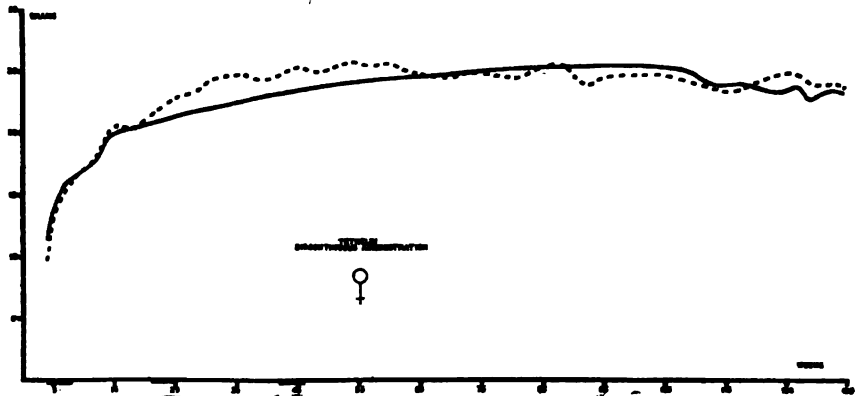


FIG. 6. COMPARISON OF THE GROWTH OF TETHELIN FED FEMALE WHITE MICE (DISCONTINUOUS ADMINISTRATION) WHICH ULTIMATELY DEVELOPED CARCINOMA (BROKEN LINE) WITH THAT OF TETHELIN FED FEMALE WHITE MICE (DISCONTINUOUS ADMINISTRATION) WHICH DID NOT DEVELOP CARCINOMA (FULL LINE)

in eight of the eleven experimental groups, the average life-duration of the group. On closer examination, however, this result is not so readily intelligible as it appears. In the first place, the carcinomatous animals display the characteristics of the long-lived animals to an exaggerated degree, and, in the second place, only a little over two-thirds of the carcinomatous animals actually belonged to the long-lived group (table 3). On the other hand, of course, many long-lived animals and, in fact, most of those which survived for the longest periods, failed to develop carcinoma at all.

We cannot, therefore, conclude that these results are due simply to the longevity induced by the superior growth-energy of the carcinomatous animals passively carrying them into the age-zone in which carcinoma occurs. A majority of all the animals attain the ages at which one-third of the carcinomatous animals die. A small minority of animals exceed the age at which the last carcinomatous animal has died, without developing carci-

TABLE 3

Showing the proportion of animals which developed carcinoma which also lived for longer than the average duration of life

CLASS OF ANIMALS	TOTAL NUMBER OF ANIMALS	NUMBER OF ANIMALS WHICH DEVELOPED CARCINOMA	NUMBER OF ANIMALS DEVELOPING CARCINOMA WHICH ALSO WERE LONG LIVED
Males			
Normal.....	32	8	6
Pituitary.....	31	9	7
Lecithin.....	32	7	5
Cholesterol.....	34	8	7
Tethelin.....	23	8	7
Totals.....	152	40	32
Females			
Normal.....	32	12	8
Pituitary.....	35	18	11
Lecithin.....	34	11	6
Cholesterol.....	32	16	9
Tethelin (discontinuous administration).....	16	4	4
Tethelin (brief administration).....	23	4	2
Totals.....	172	65	40

noma at all. Moreover, while the lowest incidence of carcinoma recorded in table 1 occurs in the shortest-lived group (tethelin, "brief administration") yet the highest incidence occurs in a group which lived an average of but twenty-four days longer (pituitary-fed females), and the next lowest incidence occurs in the longest-lived group of females (tethelin, "discontinuous administration").

A remarkable feature of the results is their uniformity. This becomes the more noteworthy when we consider the variability of the experimental material. No estimates of variability in quantitative terms have been computed, because in many cases the small number of animals which ultimately developed carcinoma in any one experimental group rendered such a computation of very little value. Nevertheless, it may readily be perceived that there is no single type to which the carcinomatous animal

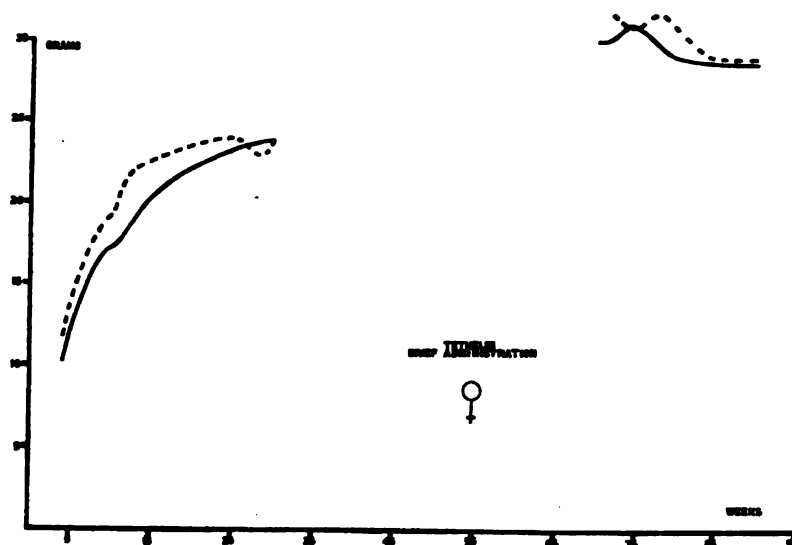


FIG. 7. COMPARISON OF THE GROWTH OF TETHELIN FED FEMALE WHITE MICE (BRIEF ADMINISTRATION) WHICH ULTIMATELY DEVELOPED CARCINOMA (BROKEN LINE) WITH THAT OF TETHELIN FED FEMALE WHITE MICE (BRIEF ADMINISTRATION) WHICH DID NOT DEVELOP CARCINOMA (FULL LINE)

adheres. No such simple rule can be formulated as that heavy animals develop carcinoma. Thus in the tethelin ("brief administration") group one of the animals which developed carcinoma weighed but 22.5 grams at seventy weeks, or nearly 7.5 grams less than the average weight at the same age of the non-carcinomatous animals of the same experimental group. Similar phenomena were observed in the other experimental groups. For this reason, of course, it is impossible to foretell the incidence

of carcinoma in any individual animal from its preceding growth. How then does it arise that the average weight of the animals which subsequently develop carcinoma is so invariably in excess of the average weight of non-carcinomatous animals throughout a large proportion of their lives?

There would appear to be only one possible explanation, namely that it is *superiority of growth relatively to the standard norm of the strain* which foreshadows carcinoma, not energetic growth *per se*. The animals employed in this, as in any other

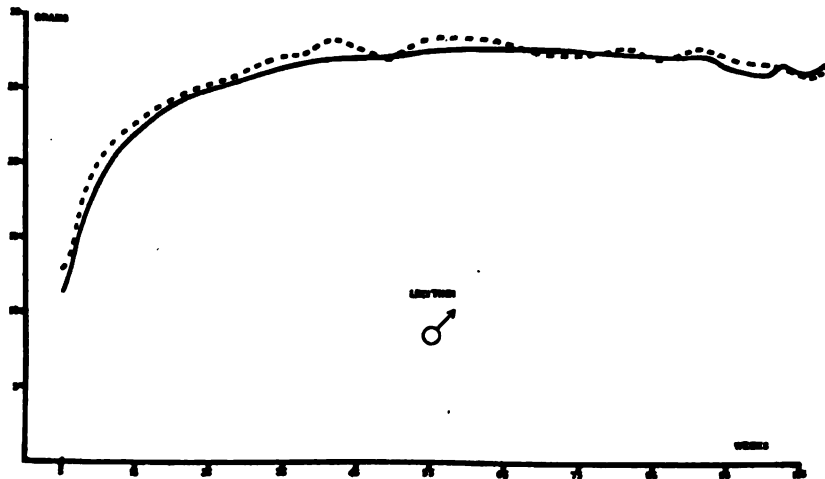


FIG. 8. COMPARISON OF THE GROWTH OF LECITHIN FED MALE WHITE MICE WHICH ULTIMATELY DEVELOPED CARCINOMA (BROKEN LINE) WITH THAT OF LECITHIN FED MALE WHITE MICE WHICH DID NOT DEVELOP CARCINOMA (FULL LINE)

animal experiment, represented a variety of more or less widely differing strains, each possessed of its own standard of absolute and relative development. In any sufficiently large group of animals chosen at random from the general stock, each of the main strains would be represented in proportion to its frequency. Since the carcinomatous animals, although so variable among themselves, invariably displayed an *average* weight which for a large period of their lives exceeded the *average* weight of the remainder, we must conclude that each of the various strains

in the stock furnished its proportionate quota to the carcinomatous group, and in each strain the animals which ultimately developed carcinoma were superior in energy of growth to the standard norm of the strain. This fact precludes the idea that we may be dealing with linked size and carcinoma-inheritance, the carcinomatous animals representing a single strain or a small number of strains distinguished also by large size. The two factors, that of absolute size and that of tendency to develop carcinoma, are, on the contrary, separable.

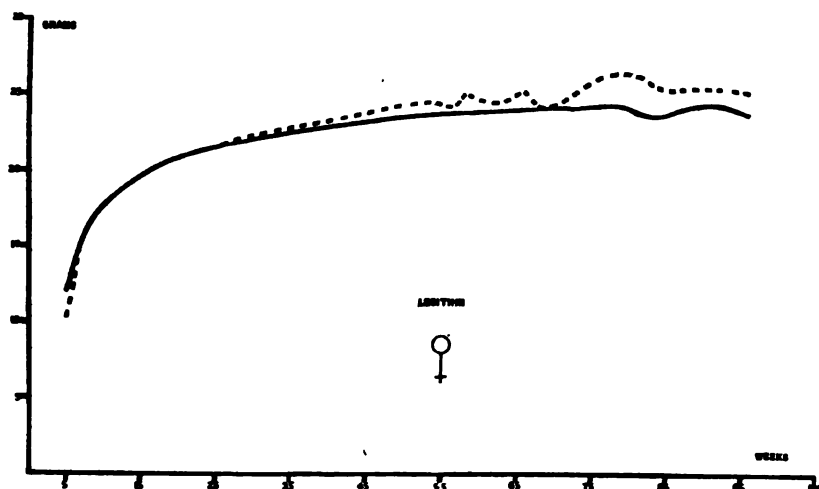


FIG. 9. COMPARISON OF THE GROWTH OF LECITHIN FED FEMALE WHITE MICE WHICH ULTIMATELY DEVELOPED CARCINOMA (BROKEN LINE) WITH THAT OF LECITHIN FED FEMALE WHITE MICE WHICH DID NOT DEVELOP CARCINOMA (FULL LINE)

In the article dealing with the comparison of the growth of long-lived with that of short-lived animals, it was sought to interpret the results in terms of the competition between parenchymatous and connective tissues during the development of the animals. In those animals in which the anabolism of parenchymatous tissues is exceptionally rapid, the growth of parenchymatous structures will be hastened and facilitated. This may or may not tend to increased weight of the animal at the time, according to the degree of acceleration experienced and the

relative masses of parenchyma and connective tissues in the body of the animal. It will, however, manifestly delay, through previous appropriation of foodstuffs, the senescent accretion of connective tissues, and consequently increase the duration of life.

If we apply this hypothesis to the results enumerated in this paper we must conclude that the animals which subsequently develop carcinoma are distinguished by exceptionally rapid anabolism and growth of parenchyma which, during adolescence, more than compensates for the complementary delay in con-

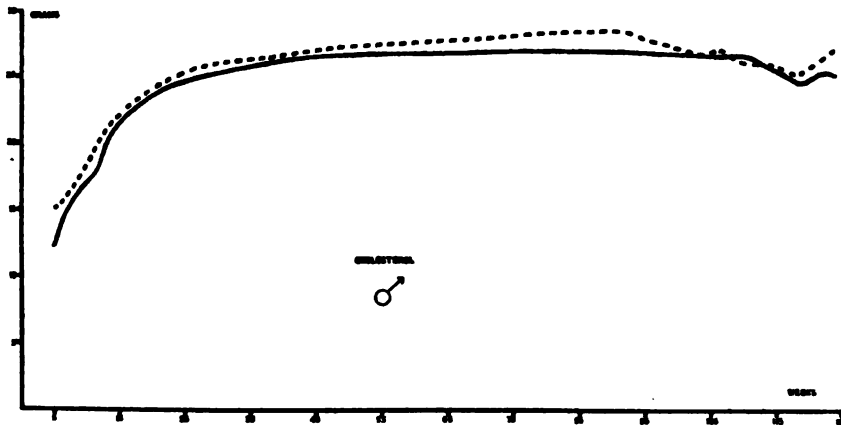


FIG. 10. COMPARISON OF THE GROWTH OF CHOLESTEROL FED MALE WHITE MICE WHICH ULTIMATELY DEVELOPED CARCINOMA (BROKEN LINE) WITH THAT OF CHOLESTEROL FED MALE WHITE MICE WHICH DID NOT DEVELOP CARCINOMA (FULL LINE)

nective tissue growth. In the absence of carcinoma this would result in great longevity, which however, is curtailed by a pathological proliferation of parenchymatous tissue. We may suppose that in such animals the energy of response to some external stimulus, such as a local source of irritation, is so excessive that when the age of the animal is sufficiently advanced to have resulted in some weakening of the competition of other parenchyma (through the parasitism of connective tissues) the effect is to institute a growth which appropriates nutriment to itself at the expense of all the adjacent tissues.

In accordance with this hypothesis we find that the incidence of carcinoma is lowest in that experimental group in which the growth curve departs most widely from the long-lived type, namely, in the tethelin "brief administration" group in which there is an extraordinary late accretion of tissue (3). The only other group of carcinomatous animals which reveals late accretion of weight is also the group which has the briefest duration of life (cholesterol-fed females, table 1).

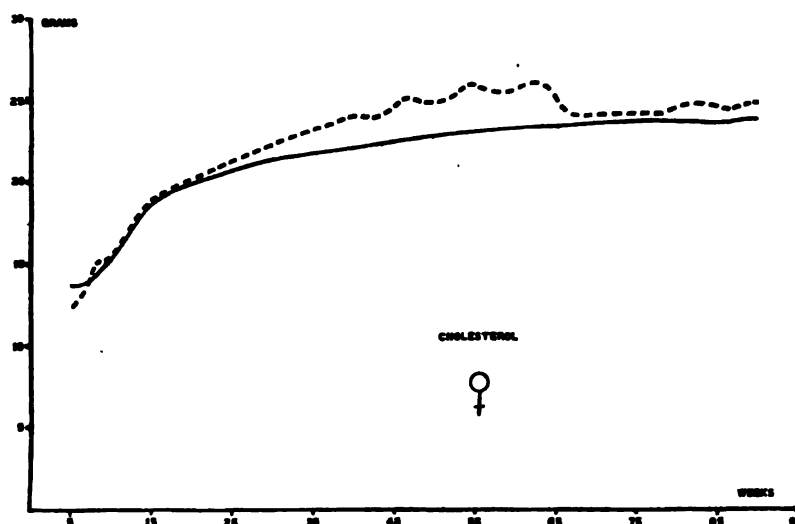


FIG. 11. COMPARISON OF THE GROWTH OF CHOLESTEROL FED FEMALE WHITE MICE WHICH ULTIMATELY DEVELOPED CARCINOMA (BROKEN LINE) WITH THAT OF CHOLESTEROL FED FEMALE WHITE MICE WHICH DID NOT DEVELOP CARCINOMA (FULL LINE)

The longest-lived animals in any group must, therefore, be those in which the speed of anabolism and energy of growth of the parenchyma just fall short of the tendency to respond abnormally, by the formation of neoplasms, to recurring local irritative stimuli.

The comparative growth of animals which ultimately developed neoplasms other than carcinoma will form the subject of subsequent communications.

SUMMARY

1. The incidence of carcinoma in mice is foreshadowed in their preceding development; but, owing to the variability of the animals, it is not possible to foretell the incidence of carcinoma in any single animal from its curve of growth.

2. The animals which ultimately develop carcinoma are distinguished by relatively energetic growth during the period of adolescence. The lead over the other animals which is thus established is usually maintained throughout life, but is sometimes lost in the later periods of life through late accretion of weight by the animals which do not develop carcinoma.

3. These results do not admit of interpretation by supposing that superior growth favors longevity, and thus passively carries the animals into the age-zone in which carcinoma occurs. A majority of all the animals surpass at death the ages at which one-third of the carcinomatous animals die. There is no correlation between the percentage incidence of cancer in any group and its average duration of life; but animals displaying an average growth curve which departs widely from the carcinomatous type also display a low percentage incidence of carcinoma.

4. The results are not due to linked inheritance of size and tendency to develop carcinoma, since the factors of absolute size and development of carcinoma are separable.

5. The results are interpreted to mean that the animals which ultimately develop carcinoma are those in which the anabolism and, therefore, the growth of parenchyma is exceptionally rapid. In such animals the energy of response to local irritative stimuli may be so excessive as to overcome the competition of other tissues and initiate a new growth.

6. The longest-lived animals in any group are, therefore, those in which the speed of anabolism and energy of growth of the parenchyma just fall short of those which lead, under the influence of recurrent irritative stimuli, to the formation of new growths.

REFERENCES

- (1) ROBERTSON, T. B., AND RAY, L. A.: *Jour. Biol. Chem.*, 1916, xxiv, 347, 363, 385, 397; 1916, xxv, 635, 647; 1919, xxxvii, 377, 393, 427, 443, 455.
- (2) ROBERTSON, T. B., AND RAY, L. A.: *Jour. Biol. Chem.*, 1920, xlii, 71.
- (3) ROBERTSON, T. B., AND RAY, L. A.: *Loc. cit.*

EFFECT OF BLOOD FROM IMMUNE ANIMALS UPON TRANSPLANTABLE TUMORS

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Even a cursory review of research in experimental cancer will reveal many inconsistencies, various workers having obtained contradictory results when attacking a problem with apparently similar methods. In many cases, however, patient repetition with careful checking up of the findings has brought to light factors which offer a satisfactory explanation for these puzzling variations. In this study an attempt was made to clear up one of the problems in which different workers have reached diametrically opposed conclusions.

Jensen (1) reported the disappearance of transplanted tumors in mice injected with serum from rabbits treated with carcinoma. In a later publication (2) he stated that while he realized that spontaneous retrogression would explain most of his "cures," it did not account for the disappearance of several of the very large tumors.

von Leyden and Blumenthal (3) reported the successful outcome of the treatment of a dog with a carcinoma, the diagnosis of the growth having been verified by microscopical examination. The method was as follows: Several rabbits were given a series of subcutaneous injections of carcinoma emulsion over a period of several weeks, and the serum obtained from these rabbits was injected subcutaneously into the dog.

Clowes (4) reported the results of experiments with mice in which one half of the animals received injections of "immune" serum obtained from mice whose tumors had retrogressed spontaneously. The other half acted as controls and received normal

mouse serum. Of the twenty mice in the first series, only one failed to show the beneficial effect of the immune serum, and all were alive at the time of the report; while in the control series, five were dead and all the others had tumors exceeding in size those of the first series. Clowes and Baeslack (5) reported the results of experiments in which one lot of mice was treated with tumor mixed with immune serum, a second with tumor mixed with normal serum, and a third with tumor mixed with normal saline solution. The difference between the results obtained in the two latter groups was negligible. The difference between the results in the "immune" set and in the two control sets, however, was considerable, 31.6 per cent of tumors developing in the controls and only 12.3 per cent in the immune group. On the basis of these findings, they justified their assumption of the presence in the blood of immune bodies antagonistic to the development of carcinoma.

Crile and Beebe (6) reported a series of ten blood transfusions in dogs with an infectious lymphosarcoma, resulting in cure in seven cases and in marked improvement in two. In the remaining case, in which the treatment was a complete failure, it was later found that the donor was not an immune animal.

von Dungern (7) obtained serum from rabbits in which there has occurred spontaneous retrogression of a sarcoma. Seven rabbits were treated by intravenous injection of this serum before being inoculated with sarcoma. Nine other rabbits were employed as controls. Six of the control animals developed tumors; in none of the treated animals did a tumor develop. Uhlenhuth, Händel, and Steffenhagen (8), however, performed the same experiment and found that rats treated with "immune" serum gave 94 per cent of successful takes, whereas those treated with normal serum gave 83 per cent, and those that received no treatment at all gave only 66 per cent. In another series of experiments, these authors mixed an emulsion of tumor tissue with immune serum. The injection of this mixture gave 100 per cent successful takes. In their experience heterologous immune serum proved just as unsuccessful as the homologous serum.

Haaland (9) treated Berlin mice with the serum of immune Hamburg mice. On subsequent inoculation of the Berlin mice with a sarcoma to which they were normally susceptible the tumors grew just as well as they did in the untreated controls. He then repeated the experiments, employing the serum from immune Danish mice; but here again there was no difference between the growth of the tumors in treated and in untreated animals.

Russell (10) and Bashford, Murray, and Cramer (11) also failed to obtain positive results with the blood from immune animals.

Sisto (12) repeated the experiments of von Dungern (13) and others in attempting to influence tumor growth by the injection of splenotoxic, orchidotoxic, and hepatotoxic sera from rabbits into which emulsions of the fresh respective rabbit organ had been injected. His published charts show practically no effect of the injections one way or the other.

It would not be amiss in a paper of this character to make mention of a closely allied but not quite similar method of treatment that has been tried in man. In 1910 Hodenpyl (14) published a preliminary report of the treatment of carcinoma by the subcutaneous and intravenous injection of ascitic fluid from a patient who, he believed, had undergone a spontaneous recovery from carcinoma. All of his patients showed subjective improvement. It is, however, well known to those who have had experience in the treatment of inoperable carcinoma that every new form of therapy along this line is followed by a temporary subjective improvement, i.e., relief of pain, diminution of weakness, improvement in appetite, etc., only to be succeeded by the inevitably fatal termination. Hodenpyl stated that the tumors diminished in size and that some disappeared. No cures were obtained, however, and the donor of the ascitic fluid also died later from cancer.

Ill and Miningham (15) repeated Hodenpyl's treatment in a series of twenty-seven cases in which ascitic fluid was injected subcutaneously. The fluid was obtained from a patient who was apparently recovering from carcinoma of the liver; but

autopsy later on revealed widespread carcinoma of the liver, ovaries, and intestines. The authors noted marked subjective improvement, although they too failed to produce a single cure with this treatment.

In the experiments about to be reported, the procedure was as follows: The rats used were those known in the laboratory as the Marshall and the August breeds. The Marshall rats are susceptible to the Flexner rat carcinoma and refractory to the Jensen rat sarcoma. The August rats have the reverse characteristics in respect to these two tumors. To make doubly sure that the animals used were immune, a preliminary inoculation of the Flexner rat carcinoma (FRC) was made in the August, and of the Jensen rat sarcoma (JRS) in the Marshall rats. Only those animals were used which did not develop tumors or in which there was a small growth with subsequent complete retrogression. These animals constituted the stock from which the immune blood was taken.

One group of Marshall rats was treated by intraperitoneal injections of blood from the August rats which had showed a natural immunity against the Flexner rat carcinoma. A second group of Marshall rats received an intraperitoneal injection of blood from normal Marshall rats. A third group received no preliminary injection of blood. All three groups were then inoculated with grafts of 0.002 gram of the FRC in the right axilla. Exactly the same plan was followed with the August rats, the immune Marshall rats being used as the source for the immune blood, and the JRS being inoculated. The amount of blood injected varied from 2 to 3 cc. depending upon the size of the animal, the idea being to take as much blood as possible without killing the rat. The tumor inoculation was made synchronously with the blood injection or within a period of forty-eight hours after it.

In accordance with the theory of the workers cited, there should have been a complete absence of growth or at least a noticeable retardation of growth in those animals that had been treated with blood taken from the immune animals. Examination of the records, however, showed that the rats treated with the immune

blood not only did not show any absence or retardation of growth of the tumor, but actually showed tumors that were in most instances larger, and developed earlier than those in the untreated animals. Even the animals treated with normal blood showed this phenomenon though in a lesser degree. Gay (16) found that injection of blood from insusceptible or refractory animals lead to an increase in the number of takes of carcinoma implantation.

Another series of experiments was made in order to determine whether the blood or other proteins injected act as a food. Three sets of rats, 36 in each group, were employed. One group received 1 cc. of blood (18 rats subcutaneously, and 18 intraperitoneally) every three days over a period of six weeks. A second group received 1 cc. of egg-white (18 subcutaneously, and 18 intraperitoneally) every three days over a similar period. A third group was kept as controls. All these animals were inoculated with the JRS, and the tumors were charted every week for eight weeks. No appreciable difference was noted in the growth of the tumors in the three groups. Hence, the possibility that the injections acted as nutriment could be disregarded.

CONCLUSIONS

It is apparent from these experiments that:

1. If immune bodies do exist in animals that are refractory to tumor growth, they are not resident in the circulating blood.
2. The transfusion of blood, if it has any influence, accelerates the development of a tumor, as regards both time and intensity of growth; the inadvisability of transfusing human cancer cases is therefore evident.
3. The results obtained by those investigators who report successful cures after injections of blood or serum must be explained by the assumption that they were dealing either with infectious granulomata or with tumors that disappeared spontaneously.

In order to reduce the effects of extraneous factors as much as possible, the tests were conducted in eight successive series, each

set containing five test animals and ten controls. The test animals were those treated with preliminary injections of "immune" blood, the controls those that received injections of normal blood or nothing at all.

REFERENCES

- (1) JENSEN: Centralbl. f. Bakteriolog., 1 Abt., Orig., 1903, xxxiv, 30.
- (2) JENSEN: Ztschr. f. Krebsforsch., 1909, vii, 281.
- (3) VON LEYDEN AND BLUMENTHAL: Deutsch. med. Wchnschr., 1902, xxviii, 637.
- (4) CLOWES: Bull. Johns Hopkins Hosp., 1905, xvi, 130.
- (5) CLOWES AND BAESLACK: Med. News, 1905, lxxxvii, 969.
- (6) CRILE AND BEEBE: J. M. Res., 1908, xiii, 385.
- (7) v. DUNGERN: Ztschr. f. Immunitätsforsch., 1910, v, 695.
- (8) HÄNDEL AND STEFFENHAGEN: Arb. a. d. k. Gsndtsamte., 1911, xxxvi, 490.
- (9) HAALAND: Berl. klin. Wchnschr., 1907, xliv, 717.
- (10) RUSSELL: Third Sci. Report, Imperial Cancer Research Fund, London, 1908, p. 341.
- (11) BASHFORD, MURRAY, AND CRAMER: Third Sci. Report, Imperial Cancer Research Fund, London, 1908, p. 315.
- (12) SISTO: Tumori, 1918, iv, 32.
- (13) v. DUNGERN: Die Antikörper, Jena, 1903, p. 30.
- (14) HODENPYL: Med. Rec., 1910, lxxvii, 359.
- (15) ILL AND MININGHAM: J. A. M. A., 1912, lix, 497.
- (16) GAY: J. M. Res., 1919, xv, 186.

INOCULATION OF SARCOMATOUS TUMORS INTO NEGRO FOWLS, WITH SPECIAL REFERENCE TO THE SIGNIFICANCE OF CHROMATOPHORES

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A kind of domestic fowl, called in western countries "silky fowl" or "negro fowl," has its origin in the Orient. Their most striking characteristics are, first, the extra toes on the inner side of the foot, showing a complete or incomplete adherence of first and second of them, and second, marked pigmentation of every part of the body. Although the feathers of genuine negro fowls are pure white or pure black, the skin itself always appears extremely bluish brown in color and contains a number of chromatophores in the cutaneous and subcutaneous connective tissue. The periosteum and endosteum, dura and pia mater, perivascular connective tissue and serosa also show the brown coloration, diffuse and spotted. Of the organs the testicles and ovaries are most strongly tinted; the lungs show spotted pigmentation as in anthracosis. The parenchymatous organs, namely, the liver, kidney, and spleen, are usually free from abnormal pigmentation. The coloration of bone is so remarkable that we suspected at first its identity with animal ochronosis.

Microscopically we ascertain that the brown coloration everywhere in the body is due to the existence of a large number of chromatophores in the interstitial tissue, especially along the course of the blood-vessels. The chromatophores assume a form quite similar to those in normal fowl, having a number of irregular long and slender processes and fine yellowish brown pigment granules with an entire similarity to melanin in microscopical appearance. Pigment granules in the cells which are in

resting stage are very fine and equal in size. When stimulated by experimental means they become irregular in size, and the cells lose their processes, taking a round shape like any other plastic cells.

The brown coloration of the bone is caused by the pigment cells in the periosteum. The endosteum of the wing bone, which covers the inner surface of empty medullary cavity, has also a number of chromatophores. The matrix of the bone is free from any kind of pigment, in contradistinction to the condition found in animal ochronosis, while the bone-corpuscles contain genuine melanin granules in the protoplasm, which cause a slight brown coloration of the bone tissue. This is especially evident in internal and external layers, near the peri- and endosteum. The bone marrow also contains chromatophores in greater or less number.

The cartilage shows generally no marked coloration. The cartilage cells, however, sometimes contain microscopically a number of pigment granules. The dura mater is deep brown like the periosteum. The pia mater shows grayish brown spots due to pigment cells. The fascia is more or less brown in color, owing to a number of chromatophores which are sometimes to be seen in intermuscular connective tissue. The periosteum, pleura, and synovial membrane also show spotted pigmentation. The pigmentation of the testicles and ovaries is caused by the existence of chromatophores in the interstitial tissue, while the tubules and the follicles have no pigment particles. A few pigment cells are found in the capsules of the spleen, liver, kidney, and pancreas, and in the perivascular connective tissue. The parenchyma of the organs and the stellate cells in the liver contain no pigment granules.

The principal subject of our investigation with this kind of fowl was to determine whether or not the chromatophores take part in the neoplastic proliferation brought about by the experimental inoculation of transplantable tumors. Such participation, if found to occur, would be undoubtedly reliable evidence of the secondary acquirement of the neoplastic properties by normal tissue. Another object was to ascertain the origin of

melanotic pigment in bone and cartilage cells in negro fowls, that is, whether it is formed in osteoblastic and chondroblastic cells themselves or is given to them by adjacent chromatophores. Moreover, we expected this material to be most suitable for the purpose of studying the relation between the pigment in epithelial cells and chromatophores, which has been an interesting subject of discussion among authors.

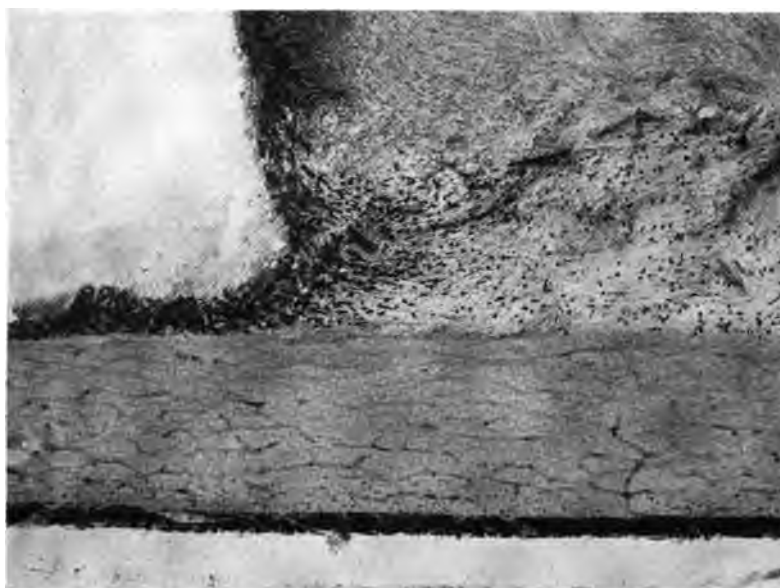


FIG. 1. Beginning myxosarcomatous growth in the endosteum of the wing bone of a negro fowl, showing a number of chromatophores in the periosteum and endosteum. The chromatophores in the endosteum are deranged and replaced by the new grown tissue. Bone corpuscles contain melanin granules, especially evident near the periosteum.

I. THE INOCULATION OF TRANSPLANTABLE TUMORS INTO NEGRO FOWLS

Two different strains of transplantable tumors (chondrosarcoma and myxosarcoma) were chosen for this purpose. Fifty negro fowls were inoculated, 15 of them with chondrosarcoma and 30 with myxosarcoma. We usually utilized for inoculation the empty medullary cavity of the wing bones, because the

endosteum is rich in chromatophores and if any proliferation should take place it could be easily followed. The wing bone was exposed, perforated by means of a borer, and inoculated with the tumor. In the case of chondrosarcoma a small piece of tissue was used; in that of myxosarcoma, except in a few instances, 2 to 4 per cent watery filtrate of the tumor extract. The tumors were easily transplantable in the medullary cavity of the wing bone. Positive transplantations were obtained in

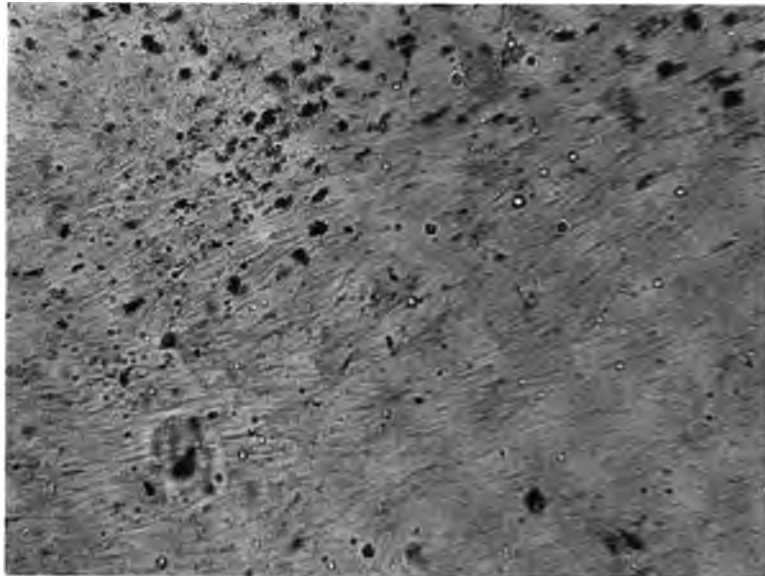


FIG. 2. Advanced myxosarcomatous tumor of a negro fowl, showing a number of melanotic tumor cells.

9 out of 15 inoculations with chondrosarcoma and nearly in all with myxosarcoma.

The development of chondrosarcoma results in a very great reactive formation of callus which consists of connective, cartilage, osteoid, and bone tissues. The neoplastic tissue, however, differs from the reactive callus tissue which is accompanied by a number of chromatophores. The chondromatous cells do not contain any particles of pigment. Briefly, in the series of inoculations of chondroma no neoplastic change of ordinary chromatophores was seen. On the contrary, the myxomatous tumor

fills up the medullary cavity of the wing bone, accompanied by some reactive callus tissue. In our cases metastasis was frequently to be seen, especially multiple, into lungs and mediastinum, exceptionally into the skin, peritoneum, pericardium, and liver. The neoplastic tissue in the medullary cavities of wing bones is somewhat gray in color. Some of the metastatic tumors also show a grayish coloration.

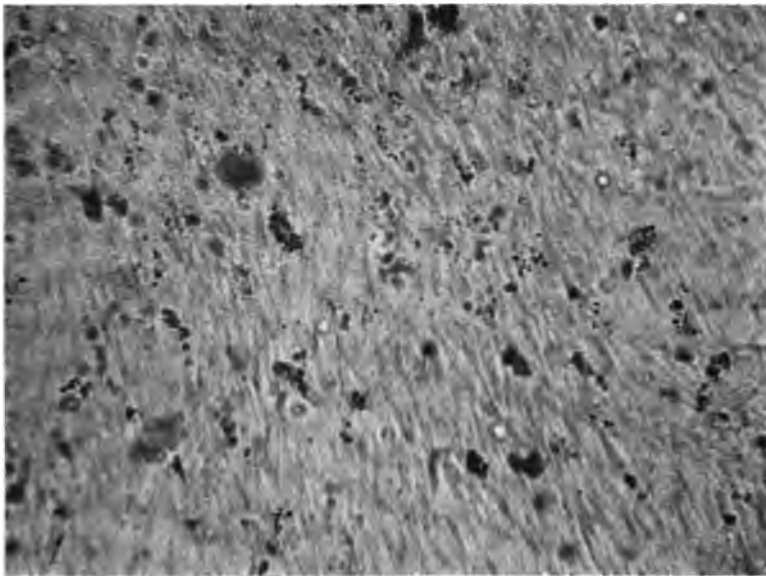


FIG. 3. The same specimen in higher enlargement.

Microscopically the neoplastic proliferation of endosteum in cases of inoculation with filtrate of tumor extract seemed to begin in the connective tissue in the layer close to the bone surface. The chromatophores in the endosteum are deranged and replaced by the new grown tissue. In this stage they did not show any sign of proliferation, having the usual form with a number of processes. Sooner or later, however, they began to multiply and produced numerous round cells with coarse pigment granules of irregular size. They were prolonged and transformed into long spindle cells which assumed an appearance quite similar to genuine tumor cells, not only in their form, but in their arrangement in the tumor tissue. Pigment granules

are generally found close to the nucleus. Most of them, therefore, are to be considered as true neoplastic elements. The pigment may not be a general metabolic product of negro fowl, for the granules are found most abundantly near periosteum and endosteum, where chromatophores exist in large number. This finding confirms that of Rous, Fujinami, Hayashi and others, that the normal tissue may acquire neoplastic nature by the

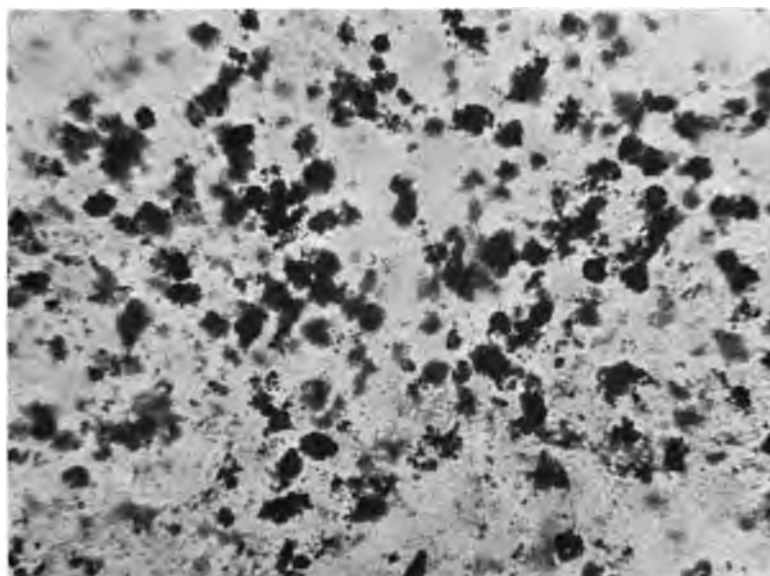


FIG. 4. Advanced myxosarcomatous tumor of a negro fowl with an enormous proliferation of melanotic cells.

inoculation of the tumor tissue or its extract. The formation of a pure melanotic tumor is not to be expected, for the pigment cells are always accompanied by a large number of the usual connective tissue cells, which are more readily proliferative than the former. In further generations of inoculation of tumor tissue with melanotic elements into the normal fowls the pigmented tumor cells gradually disappear and the neoplastic proliferation of neighboring tissue cells overcomes the transplanted pigmented tumor cells.

II. THE RELATION BETWEEN THE MELANOTIC PIGMENT IN EPITHELIAL CELLS AND CHROMATOPHORES

With regard to the origin of the melanotic pigment in chromatophores and in epithelium a difference of opinion exists. Some authors maintain that the pigment originates in chromatophores and then goes over into epithelium, while others assert that it is primarily produced in epithelial cells. At the present time a majority of authors seem to consider that melanotic pigment is formed independently both in epithelium and in chromatophores.

The negro fowl offers a most suitable characteristic for the purpose of solving this problem. As mentioned, there are two kinds of negro fowl, white feathered and black feathered. Melanin granules are not to be found in the epithelium of the white feathered, while the black feathered variety has a number of pigment granules in the epithelial cells of the skin, more abundantly in the exposed parts such as the legs or the crest. In other parts of the body, where stratum corneum is thick, the melanin content of epithelium is generally light. The chromatophores in cutaneous and subcutaneous connective tissues differ in numbers among both kinds of negro fowls. In this point the white feathered negro fowl is to be compared with the ape (*Cercopithecus mona*), which has white hair and no pigment in the skin, but a large number of chromatophores in the cutaneous connective tissue. On the other hand the embryonal skin of the normal black feathered fowl shows the pigment only in the epithelial layer in the early stage. We recognized, therefore, that melanin pigment may be formed in both elements independently. The migration of the pigment granules from one to another seems to be disproved in this case, although the general idea that the pigment of the eye is formed in the retina and secondarily given to the cells of the choroid, may not be impossible.

III. THE ORIGIN OF MELANOTIC PIGMENT IN BONE CORPUSCLES AND IN CARTILAGE CELLS

It is rather important to determine whether the above mentioned pigment in bone and cartilage cells is formed in the cells themselves or is given from the adjacent chromatophores. For this purpose I have intentionally broken the ulna of negro fowls in one or both sides and examined the callus histologically in the course of one to forty-three days after fracture. Twelve negro fowls were used for this experiment. During healing of fractured bones the callus formation takes place at first close to the bone tissue, stripping off the layer of chromatophores. Periosteal chromatophores are arranged close to each other in the earlier stage. With the advance of callus formation they are separated by new grown tissue cells. Actual proliferation of chromatophores takes place in some degree, forming round shaped cells with irregular melanin granules. At the same time melanin granules are to be found in some typical osteoblasts and chondroblasts in the callus, but far less in number than in new formed chromatophores. Pigment granules in the plastic cells are generally coarse in size, but they do not show any microchemical difference from those in chromatophores. The amount of pigmented plastic cells is generally proportional to the number of chromatophores in the body. There is no actual sign of transference of pigment from chromatophores to plastic cells. The metaplastic change of chromatophores to fixed tissue cells is not recognizable, for the latter are to be acknowledged undoubtedly as a sort of wandering cell. We conclude, therefore, that genuine osteoblasts and chondroblasts themselves can make melanin into protoplasm, and that the melanin formation may occur in many kinds of tissue cells, if there are some melanogenetic compounds from which melanin could be derived, and fermentative (perhaps oxidative) elements which act on them.

SUMMARY

1. Oriental negro fowls have a large number of chromatophores in all parts of the body, especially in the connective tissue, dura

and pia mater, periosteum and endosteum, serous membrane, interstitial tissue of genital glands, and in the lungs.

2. The bone corpuscles and occasionally the cartilage cells also contain melanotic pigment in their protoplasm.

3. Myxosarcomatous tumors of negro fowls grown by inoculation of tissue or its extract assume a melanotic nature, probably due to the neoplastic proliferation of chromatophores. This is actual evidence for the acquisition by normal tissue of neoplastic nature.

4. The melanotic pigment in the epithelium and connective tissue is formed in both elements independently of each other. The migration of pigment granules from one to another is not recognizable.

5. Osteoblasts and chondroblasts in callus also form melanotic pigment in their protoplasma.

INFLUENCE OF THE LYMPHOCYTE ON THE PERITONEAL IMPLANTATION OF SARCOMA IN MICE

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No phase of tumor investigation in recent years has aroused greater interest than that of the relation of the lymphocyte to natural and induced resistance to cancer. Following the observation by Da Fano (1) that lymphoid and plasma cells are constantly associated with degenerating tumors and that the lymphocyte might be the agent which distributes immunity throughout the organism, many articles have been written tending to prove an antagonistic action between the lymphocyte and tumor growth. Burgess (2) noted that the retrogressing implant of cancer in mice is invariably surrounded by large numbers of lymphocytes. Similar observations were made by v. Dungern and Coca (3). Apolant (4) found increased difficulty in immunizing splenectomized animals to tumor implants. Tyzzer (5) working with a Japanese waltzing-mouse tumor found that implants grew vigorously for a time in non-susceptible mice, but were destroyed soon after by an inflammatory reaction excited only by living tumor; the cell infiltration disappearing as the tumor became necrotic. In previously immunized non-susceptible mice the process of destruction was the same but began much earlier. Braunstein (6) concluded that the spleen possesses highly developed powers of resistance against tumor growth, while splenectomized animals are more liable than normal animals to inroads of malignant growth. Rohdenburg and Johnston (7) made parallel observations upon thymus, pancreas, spleen, hypophysis, and testis, showing that after extirpation of the thyroid, thymus, or testes, the animal had a lessened resistance to carcinoma.

Baerslack (8) studied the blood of inoculated animals and reported a decrease in the numbers of circulating lymphocytes in mice with growing tumors. Murphy (9), working with tissue cultures, noted that resistance to heteroplastic tissue grafts apparently depends upon the activity of the lymphocyte and obtained similar results with experimental cancer in mice. Murphy and Morton (10) in a series of experiments with transplanted and spontaneous tumors in mice noted that slight exposure to Roentgen rays, inducing a lymphocytosis, brought about a more highly resistant state to implanted tumor; whereas larger doses of the *x*-ray, inducing a leucopenia, caused a decreased resistance to inoculation with tumor, and that animals naturally immune were rendered susceptible by this method. They concluded that destruction of the lymphocytes by the *x*-ray causes a loss of the natural or induced resistance to the growth of inoculated cancer.

The apparently conclusive results of the investigators referred to above have not been without contradiction. Wade (11) noted that with the establishment and growth of an inoculable tumor, there is a steady increase in the percentage of lymphocytes in the circulating blood. Rous and Murphy (12), experimenting with sarcoma of the fowl, decided that no fundamental importance can be attributed to the protective function of lymphocytes in mammals and birds alike although they did state that the "lymphocyte has an association with the process of resistance in the fowl more marked than that seen in mammals."

Serafini (13) observed that ligation of the splenic vessels in rats and the injection of splenic tissue does not promote the growth of implanted tumor. Jones and Rous (14) noted that mice inoculated eight to ten days after splenectomy have the same resistance as normal animals; and that mice inoculated three and a half weeks after splenectomy live longer than normal animals. Bullock (15) and Stevenson (16) could not find that the presence of spleen inhibited the growth of tumor implanted into the chick embryo. Sittenfield (17), having utilized various means of inducing a hyperlymphocytosis, stated that "neither increase nor reduction of the lymphoid elements in the blood

had any influence upon either resistance or susceptibility to tumor growth." Stevenson (18), again, found in 1917 that tumors show no inhibition of growth when grown on the allantois of the chick embryo for ten days in the presence of chicken spleen. Bullock and Rohdenburg (19) (20) also reported that removal of the spleen does not favor the growth of heteroplastic tumor grafts. More recently Prime (21) found that, following lymphocytic reduction by exposure to the x-ray, no appreciable decrease in immunity to tumor inoculation occurs.

As a result of these conflicting and widely divergent results we find that the rôle of the lymphocyte in immunity to cancer is still undetermined. The varying conclusions arrived at are due to the methods employed and to the instability of the leucocyte count in the lower mammals, for blood-cell counts in mice may be extremely variable. In attempting to devise a new means of approaching the problem, it occurred to the author that intraperitoneal inoculation of a tumor, preceded and followed by study of the cellular elements of the peritoneum, might yield information regarding the part played by the lymphocyte in cancer immunity.

The normal peritoneum of the white mouse contains numerous cellular elements, among which are found in predominating numbers the large mononuclear phagocytic cells designated as macrophages and small mononuclear cells having the morphology and staining reactions of lymphocytes. In addition there are large cells whose cytoplasm is filled with coarse basophile granules, resembling the mast cells of the blood, a few polynuclear leucocytes, and erythrocytes rarely. The macrophages, as nearly as can be determined by direct counts, are found to vary from 10,000 to 20,000 per cubic millimeter, whereas the lymphocytes are far more numerous, usually about 115,000 per cubic millimeter. In a few mice the macrophages predominate, but in the majority the lymphoid cells are more numerous. In differential counts made by obtaining peritoneal fluid with capillary glass pipettes, the average percentages found in a series of 24 mice were as follows:

	<i>per cent</i>
Macrophages.....	34.3
Large mononuclears.....	7.0
Lymphocytes.....	55.0
Po'ymorphonuclear neutrophiles.....	3.5
Basophi es.....	0.2
	<hr/> 100.0

The large mononuclears in the above classification are much larger than the lymphoid cells, but do not possess the abundant foamy cytoplasm of the macrophage. The designation of the small mononuclear cells as lymphocytes may be questioned. Evans (22) apparently refers to certain small mononuclears in the peritoneum as young macrophages, asserting that they will take up granules of vital stains, a characteristic lacking in true lymphocytes. This may apply to the cells referred to above as large mononuclears. Morphologically we find the small cells to be identical with the lymphoid cells of the blood. We do not find that they possess phagocytic properties for bacteria, inert particles, or vital stains; and finally, they decrease markedly under x-ray exposures.

It is very evident, then, that the peritoneum of the mouse contains large numbers of cells having all the characters of lymphocytes, and it is the purpose of the present investigation to show their influence on intraperitoneal inoculations of a mouse tumor obtained from the Crocker Research Fund, where it is designated as no. 180, and is regarded as a sarcoma. The tumor grows readily, and in our hands has not failed in more than 5 per cent of subcutaneous and 2 per cent of intraperitoneal inoculations. Inoculations were made by means of a small trocar needle.

The white mice selected were young adult animals; an equal number of each sex. Smears from the peritoneum were made before inoculation and repeated after inoculation till the "taps" became bloody and unfit for differential counting. Considerable variation in the percentage of lymphocytes occurred in the inoculated mice, and also some variation in the same mouse at different times. The lowest differential count recorded showed 25 per cent lymphocytes, the highest 80 per cent of lympho-

cytes, and the average in uninoculated mice 56 per cent lymphocytes. After inoculation, when the tumors were well developed and readily palpable through the skin, there was found an average of 54 per cent of lymphocytes. Twenty-two mice were used in this experiment. About half the animals showed a rise and the other half a fall in the percentage of lymphocytes following tumor inoculation. Table 1 illustrates the results

TABLE 1

MOUSE	PERCENTAGE OF LYMPHOCYTES		TUMOR
	Before inoculation	After inoculation	
18	60	75	Large
19	50	74	Large
20	30	56	Large
21	48	45	Large
24	80	75	Large
27	66	75	Large
30	60	64	Large
32	64	45	Large
33	65	85	Large
34	44	56	None
35	61	32	Large
36	54	40	Large
37	50	29	Large
47	56	53	Large
67	48	50	Large
68	66	70	Large
69	44	58	Large
70	49	31	Large
71	60	50	Large
74	80	70	Large
76	46	29	Large
77	40	42	Large

Of 22 mice, 11 show an increase in lymphocytes, and 11 a decrease.

obtained before and after inoculation. Following the inoculation, numerous polymorphonuclear leucocytes appeared in the peritoneum but not in sufficiently large numbers to influence visibly the percentages of the other cellular elements.

Thus, as indicated in the table, of 22 mice inoculated with tumor all with one exception presented in a short time (ten to twenty days) rapidly growing tumor nodules. In making the

punctures with capillary glass tubing blood was frequently obtained and when abundant the slides were discarded. The counts recorded above were made on relatively blood-free preparations. Since many mice showed an increase and others a decrease in the numbers of lymphocytes, and since the tumors were large and growing rapidly, it is apparent that no marked adverse influence can be attributed to the presence of the lymphocytes. The most important point brought out by the present investigation is the fact that small fragments of tumor tissue placed in a fluid rich in lymphoid cells showed no inhibition of growth.

CONCLUSIONS

1. The normal peritoneum of the white mouse is rich in cellular elements—115,000 to the cubic millimeter—of which about 55 per cent are lymphocytes.
2. Mouse tumor implanted into the peritoneum of these mice grows rapidly.
3. No marked change in the cellular content of the peritoneum of such mice results from the inoculation of mouse tumor.
4. No direct antagonistic action is found to exist between the lymphocyte and tumor implants in mice.

REFERENCES

- (1) DA FANO: *Ztschr. f. Immunitätsforsch., Orig.*, 1910, v, 1.
- (2) BURGESS: *Jour. Med. Res.*, 1909, N.S., xvi, 575.
- (3) v. DUNGERN AND COCA: *Ztschr. f. Immunitätsforsch., Orig.*, 1909, ii, 391.
- (4) APOLANT: *Ztschr. f. Immunitätsforsch., Orig.*, 1913, xvii, 219.
- (5) TYZZER: *Jour. Med. Res.*, 1915, N. S., xxvii, 201.
- (6) BRAUNSTEIN: Quoted by Oser and Pribram, *Amer. Jour. Med. Sci.*, 1913, cxlvi, 310.
- (7) ROHDENBURG AND JOHNSTON: Quoted by Oser and Pribram, *Amer. Jour. Med. Sci.*, 1913, cxlvi, 310.
- (8) BAESLACK: *Ztschr. f. Immunitätsforsch., Orig.*, 1914, xx, 421.
- (9) MURPHY: *Jour. Exper. Med.*, 1913, xvii, 482.
- (10) MURPHY AND MORTON: *Jour. Exper. Med.*, 1915, xxii, 204, 800.
- (11) WADE: *Jour. Path. and Bacteriol.*, 1908, xii, 384.
- (12) ROUS AND MURPHY: *Jour. Exper. Med.*, 1912, xv, 270.
- (13) SERAFINI: *Tumori*, 1917, v, 229.
- (14) JONES AND ROUS: *Jour. Exper. Med.*, 1914, xx, 404.

- (15) BULLOCK: *Lancet*, London, 1915, i, 701.
- (16) STEVENSON: *Jour. Cancer Res.*, 1917, ii, 245.
- (17) SITTENFIELD: *Jour. Cancer Res.*, 1917, ii, 151.
- (18) STEVENSON: *Jour. Cancer Res.*, 1917, ii, 449.
- (19) BULLOCK AND ROHDENBURG: *Jour. Cancer Res.*, 1917, ii, 465.
- (20) BULLOCK AND ROHDENBURG: *Jour. Cancer Res.*, 1918, iii, 31.
- (21) PRIME: *Jour. Cancer Res.*, 1920, v, 105.
- (22) EVANS: *Amer. Jour. Physiol.*, 1915, xxxvii, 243.

PLATE 1

FIGS. 1 AND 2. Examples of rapidly growing peritoneal tumors following tumor inoculation into mice with numerous lymphocytes in the peritoneum.



FIG. 1



FIG. 2

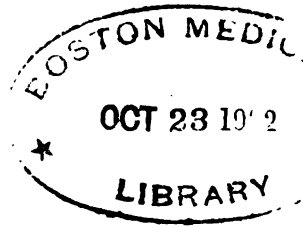


PLATE 2

FIGS. 3 AND 4. Peritoneal smears from normal mice. Obj. 16 mm. Oc. 5. Note the large numbers of lymphocytes. The larger pale-staining cells are macrophages. A few mast cells are present.

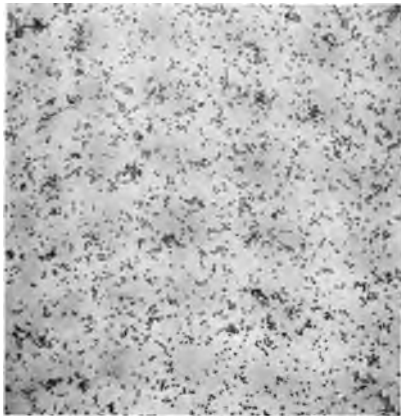


FIG. 3

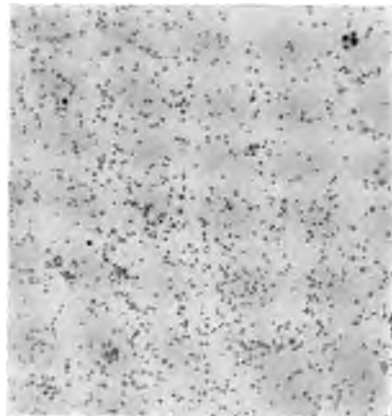


FIG. 4

PLATE 3

FIG. 5. Peritoneal smear showing large numbers of macrophages. Tumor growth rapid. Obj. 4 mm. Oc. 5.

FIG. 6. Peritoneal smear from mouse which showed 75 per cent of lymphocytes in presence of well-developed peritoneal tumor. Obj. 4 mm. Oc. 5.

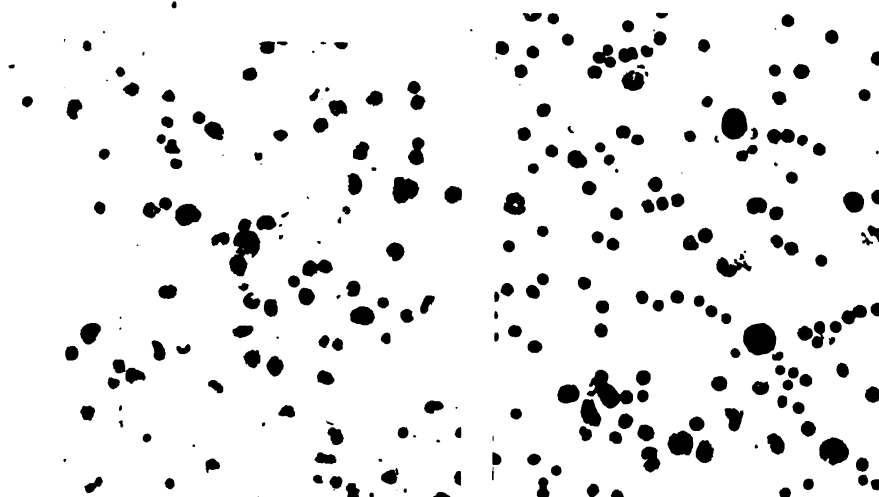


FIG. 5

FIG. 6

PLATE 4

FIG. 7. Peritoneal smear showing 75 per cent lymphocytes. Tumor large and rapidly growing. Obj. 4 mm. Oc. 5.

FIG. 8. Peritoneal smear showing 70 per cent lymphocytes. Tumor large. Obj. 4 mm. Oc. 5.

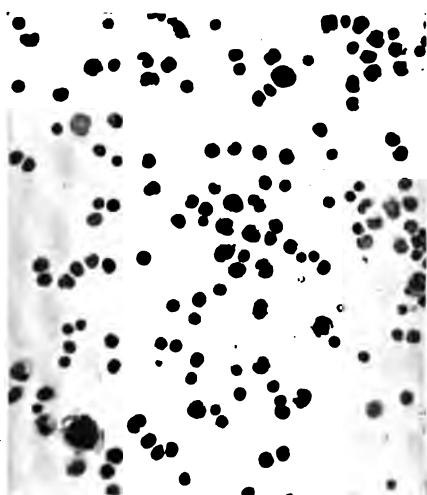


FIG. 7

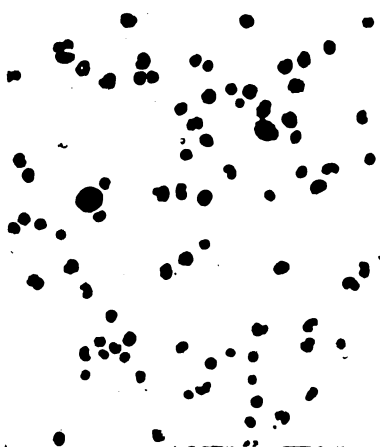


FIG. 8

PRIMARY SPONTANEOUS SQUAMOUS CELL CARCINOMAS IN MICE

STUDIES ON THE INCIDENCE AND INHERITABILITY OF SPONTANEOUS TUMORS IN MICE

FIFTEENTH COMMUNICATION

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Throughout most of the animal kingdom primary carcinoma of the skin is met with but rarely, in contrast with its great frequency in man, and also with the relative frequency of other cutaneous and subcutaneous tumors in animals. The existing literature on spontaneous tumors in animals indicates that no species except dogs, horses, and fowls, show skin carcinoma except as a rare condition, and this is of particular significance in view of the fact that skin carcinomas are more certain to be recognized than those in any other location and hence their relative infrequency is even more marked than any statistical evidence would indicate. Presumably the unprotected condition of the human skin accounts for its susceptibility to cancer, as the hairy scalp rarely shows primary carcinoma, and the pigmented skin of the negro also seems insusceptible.

The classical compilation of Sticker (1), which contains records of many tumors not fully differentiated according to modern criteria, gives the following relative frequency in this material of skin tumors in different species:

	SPECIES				
	Horse	Cattle	Dog	Cat	Swine
Total carcinomas.....	332	78	766	21	12
Skin.....	22	2	166	6	2
Eye.....	14	2	8		
Lip.....	4	1			
Anus.....	8		89	1	
Penis.....	52	2	16	1	
Vulva.....	11	1			

The extensive compilation by Teutschlaender (2) of the distribution of tumors in animals shows that cases of squamous cell carcinoma have been described in but few species, according to his records, namely: cat, dog, mouse, rat, elephant, beef, sheep, pig, horse, mule, cockatoo, toucan, chicken, carp, and gold fish. However, in another table he mentions also a case in a stag.

Apparently birds develop cutaneous carcinoma somewhat more often than most other animals, but even here there are but few recorded. In their summary of the literature on tumors in birds, Joest and Ernesti (3) found but 4 of 37 tumors described in birds to be skin carcinomas, those being two found on the legs of chickens by Wernicke; one on the wing of a "papapeis"—reported by Guerrini, and one on the wing of a toucan reported by Herbert Fox (4), this last case exhibiting also a metastasis in the lung. In none of their own 50 cases of tumors in birds was a skin carcinoma described. This compilation, as well as all others that we have observed, omits the striking case of squamous carcinoma of the skin of the foot of a hen described by Boynton (5), in which transplantations were made without success. More recently a similar tumor arising on the thumb claw of a lark has been reported by Urrea (6). Two cases of squamous cell carcinoma of the mouth in fowls have been described by Pick and Koch. The experience of Teutschlaender (7) is unique, for he reports that of 54 carcinomas in fowls observed in the cancer laboratory at Heidelberg, 28 were skin epitheliomas, of which 18 were on the foot, attributed to the irritation of parasitic infection of the epidermis (*Cnemidocoptesmilben*).

Apparently fish are subject to skin tumors of various structure, including carcinoma. Thus, Fiebiger (8) reports cases of epithelioma occurring on the lip of each of two "Schlei" (*Tinca vulgaris* Cuvier) coming from the same pond, a markedly malignant skin epithelioma in a carp, and mentions papillomatous growths in several specimens of Kletterfish (*Anabus scandens*). Plehn has also described numerous skin epitheliomas in cyprinoid fishes.

Bashford has reported a squamous cell carcinoma in a small fish, *Gasterosteus spinachia*, two cases of carcinoma of the skin in frogs, and a carcinoma of the skin glands in a Triton.

Among mammals the dog alone seems to be commonly subjected to skin carcinoma, if we except the carcinoma of the penis and prepuce occurring not infrequently in horses and oxen. It would seem to be not uncommon in cats according to the experience of Roffo (9), who found 7 tumors in 307 cats examined, of which 4 were skin carcinomas on the head and face, with lymph gland metastases, but none of 11 cases of tumors in cats collected by Bashford (10) was of this type, and but 2 of 48 tumors in dogs, although there were several squamous cell growths in the mouth and pharynx. Leo Loeb (11) reported in 1903 that from one to three carcinomas of the inner canthus of the eye were observed every week in the Chicago stock yards, but in one year no other external carcinomas were observed except two of the vulva.

Rodents seem to have skin carcinoma but rarely. The large series of wild rats autopsied in plague work and examined for tumors (McCoy, Woolley and Wherry, Beatti) have revealed no such growths except for an epithelioma of the vulva reported by Woolley and Wherry (12). It will be recalled that the early transplantation experiments of Hanau (13) were with a squamous cell carcinoma from the vulva of a white rat, and he states that there had been two previous cases of similar growths in rats in their laboratory. Roffo (14) has also observed a squamous cell carcinoma which arose in the vulva of an old white rat, which was transplanted through six generations without change in structure. In view of the great frequency of parasitic skin infection in rats,

"rat scabies," commonly with extreme papillomatous proliferation of the epithelium (15), it is strange that epithelioma has not been more frequently observed. Of 123 cases of spontaneous rat tumors reported in the literature and collected by Bullock and Rohdenburg (16), there were but three epitheliomas, one of the tongue and two of the vulva. They reported on 48 rat tumors observed in the Crocker Laboratory, one of which was an epithelioma of the head, which was not described further. Morris (17) has reported a basal cell carcinoma which arose in the skin of a male rat, five months old, and which died out after two generations of transplants. He was unable to find other recorded instances of basal cell carcinomas in animals.

Several instances of squamous cell carcinoma have been described in mice, but they form an insignificant proportion of the many spontaneous tumors that have been observed in this species. Nevertheless they were looked upon as of great significance in the early days of experimental cancer research, since they established the fact that mice had other forms of tumor than the predominating mammary gland carcinoma. Haaland (18) reported a case of squamous cell carcinoma arising in the mouth of a mouse in Borrel's laboratory, involving the lower jaw, showing a typical squamous cell structure. Six mice were inoculated from this, but as in six months they showed no growths they were put back into the cage with the others. Ten months after the inoculations "*deux cas identiques de cette même tumeur apparurent en même temps dans cette cage,*" but it was not known whether these were inoculated mice or not and Haaland does not make clear whether these later tumors were in the mouth, although this is apparently what he means. Later he observed a third case of mouth cancer in a mouse recently inoculated with a Jensen tumor. No growths developed in mice inoculated from this mouth tumor.

Murray (19) in 1908 described the following cases of superficial squamous cell carcinoma in mice: (1) A prickle cell growth with apparently little keratinization, arising in the skin of the neck, with metastasis into an adjacent lymph gland. (2) A carcinoma presenting both alveolar and squamous cell areas,

with keratinizing metastases in the lung. Presumably this tumor arose in the mammary gland. (3) Prickle cell growth without keratinization, interpreted as primary in the nipple; this mouse also had had a hemorrhagic adenocarcinoma of the mammary gland removed by operation. In discussing the mammary gland tumors he also describes the occurrence of areas of keratinization within tubular carcinomas, and regards them as indications of the close association of the mammary apparatus with the skin from which it develops, and points out that the ampulla which receives the terminal portions of the mammary ducts is also lined by stratified squamous epithelium. "Therefore, should the cells of the new growth have taken their origin near the nipple, variations in either direction are only to be expected."

In his later report from the London laboratories, Haaland (20) states that of 353 spontaneous tumors observed (not including those in Murray's report), 22 were squamous-celled carcinomas with marked keratinization, of which 14 arose in the mammary region and 8 outside it. Of these 8, 3 were mouth tumors similar to those observed in Borrel's laboratory, apparently arising in the mucous membrane of the inside of the cheek, there being in one metastases in the lymph nodes. One seemed to have arisen on the surface of the head, and it penetrated the skull; grafts from this were successful. (The illustrations of these tumors show the gross relations of such tumors so well that we are spared the necessity of reproducing further illustrations.) Three tumors were described as superficial verrucous growths, on the vulva, anus, and left flank. One was a typical squamous cell carcinoma of the skin of the chest, developing as a superficial ulcerating growth, "undoubtedly from the skin itself, or from the mamilla." A case of mixed squamous and sebaceous carcinoma of the vulva is also mentioned.

Several of the keratinizing mammary tumors differ from typical squamous cell carcinomas, and their cutaneous origin is doubtful. Four are described as resembling "molluscoid" tumors, characterized by long radiating cylinders of cells with a central keratinized zone, found in the mammary region and not giving successful transplants. Four tumors are described as "typical

squamous-celled alveolar carcinomata in the mammary region." Six others are called "adenocarcinoids," these being unquestionably mammary gland adenocarcinomas in which areas of keratinization occur. Attention is called to the occurrence of squamous-celled cysts in the normal mamma, apparently arising from mammary ducts in which the epithelium has become metaplastic, and which perhaps explains the occurrence of keratinizing carcinoma in the mammary gland. As squamous-celled growths have also been found rarely in the human breast, as well as in cats and dogs—Teutschlaender (21)—the disease in mice is not without analogy, although apparently relatively frequent among the mammary gland tumors of this species. Presumably in some stocks of mice keratinization is even more common in mammary gland carcinoma than the above reports indicate, for Woglom (30) found this change in 228 of 1000 spontaneous mammary gland carcinomas examined in the Crocker Laboratory.

Pick and Poll (22) reported as a "sweat gland carcinoma" a tubular growth arising near the scapula of a mouse, but the description published does not permit the exclusion of a mammary gland origin.

Erdheim (23) described a pedunculated tumor, which gave no evidence of malignancy, but exhibited a histological structure resembling squamous cell carcinoma. It arose from the vulva of a mouse, and after the outer part of the growth had been removed the base healed spontaneously. He was unable to classify this tumor, beyond indicating that it was a stratified epithelial neoplasm.

Tsutsui (24) has produced carcinoma on the skin of the back of mice by painting with tar, after the procedure used by Yamagiwa with the ears of rabbits, observing 16 carcinomas and 1 sarcoma in 17 mice surviving over 100 days. In two cases lung metastases were observed.

We find no other reports of squamous cell carcinoma arising in the skin or mouth of mice, which indicates their relative infrequency, since such tumors are most obvious when they do occur. Thus, Tyzzer (25) in his report of 83 spontaneous

tumors in mice includes no tumors of this type. In the 41 primary mouse tumors described by Jobling (26) there were no skin epitheliomas, although there was one of the molluscoid keratinizing tumors such as Murray, Haaland, and Tyzzer have described.

Squamous cell carcinomas outside the mammary gland and skin of mice are rare. Tyzzer reported that among his lung tumors "in several cases" the growth was of an epidermoid character, and Haaland described one such tumor. In our previous paper on primary carcinoma of the stomach in mice (27), we collected four cases of squamous cell gastric carcinoma reported in the literature and added three more in our own material, as well as one case of squamous carcinoma in the external surface of a chronically prolapsed rectum. Fiebiger (31) has also reported the occurrence of a few instances of squamous cell carcinoma produced experimentally in the stomach of mice by feeding cockroaches injected with *Spiroptera*. We have found no reports of squamous cell carcinomas arising in the esophagus or urinary tract, or in the cervix uteri, or in any other structure where they might be found in mice, except such as have been mentioned above.

OBSERVATIONS ON SKIN TUMORS AND SQUAMOUS CELL CARCINOMAS IN THE SLYE STOCK OF MICE

In 28,000 consecutive autopsies performed on mice of this stock, which had been permitted to live as long as possible without any experimental manipulations whatever, we have observed the following instances of primary neoplasms of squamous or stratified epithelial structure.

Primary squamous cell carcinoma of the skin and mouth	70
Primary basal cell carcinoma of the skin	15
Primary keratinizing carcinoma of the mammary gland	56
Primary squamous cell carcinoma of the stomach	4
Primary keratinizing carcinoma of the lung	1
Primary squamous cell carcinoma of the rectum	2
Primary squamous cell carcinoma of the vagina	1
Primary stratified carcinoma of the meibomian gland	2
Primary sebaceous gland adenocarcinoma	1

As we have not yet made a complete analysis of all the tumors observed in these 28,000 autopsies the proportion of stratified cell tumors to total tumors cannot be stated. Roughly there are probably about 4000 primary spontaneous tumors of all sorts, so that these squamous cell tumors constitute not far from 4 per cent of the total.

CARCINOMA OF THE SKIN

In our material are 85 malignant epithelial growths arising in the skin, mouth, and lips, excluding those squamous cell growths that seem to be derived from mammary gland tissue and stratified cell growths from other glands. Of these, 70 are of squamous cell type and 15 of basal cell character. Since many of the tumors which arise about the lips and mouth are, when observed, so extensive that their exact point of origin cannot be determined, we have grouped them together with the skin tumors. Roughly classifying the site of the 70 squamous cell growths, 13 were in the skin of the trunk, one on a front limb, 2 on the vulva, 15 about the lower jaw, 18 on some other portion of the face, and 23 about the ears or neck; that is, all but 16 of the 70 were on the head and neck. Presumably this is to be explained by the much greater amount of traumatism suffered by the skin in this part of the body, which is especially marked in cage mice which are always rubbing their muzzles against the rough wire meshes of the cages. The tumors of the jaw and mouth in several instances seemed to have resulted from the irritation produced by broken or protruding teeth. Nearly all of the carcinomas arising on the trunk were definitely located at the site of a healed wound, and in not a few of those of the head the same origin was observed; it is probable that less obvious wounds are responsible for many if not all of the others. A chronic dermatitis often preceded the carcinomas of the skin of the face, presumably incited by traumatism. The not uncommon fungus infections of the skin seem to be too rapid in their course to lead to carcinoma formation.

Clinically these growths are similar to corresponding forms of carcinoma in man (fig. 1). The age at which they occur is, on an average, later than with any other mouse tumors, as they rarely appear before late middle life, and usually only in old age. They arise under crusted ulcers, or develop in areas of hyperplasia from chronic irritation, spread slowly as crusting, ulcerated growths, destroying at times the eyes or other features, usually remain superficial, and most often cause death by chronic infection of the ulcerated surface, which may also lead to acute



FIG. 1. HEAD AND FACE OF MOUSE WITH EXTENSIVE BASAL CELL CARCINOMA OF THE SKIN, OF AT LEAST FIVE MONTHS DURATION. No. 7874

abscess formation. Occasionally death results from starvation when the cancer involves the mouth or jaw, from hemorrhage, invasion of the skull, or other coincident diseases.

The microscopic diagnosis of the skin neoplasms is often difficult, especially with the basal cell growths, since we find all stages of epithelial overgrowth from simple hyperplasia to metastasizing carcinomas. In this series no growth is included as a carcinoma unless it exhibited both the gross and clinical features of malignancy together with distinct microscopic evidence of infiltrative character. The extensive destruction of these growths by infective processes often obscures the microscopic findings. In several instances a growth that presented

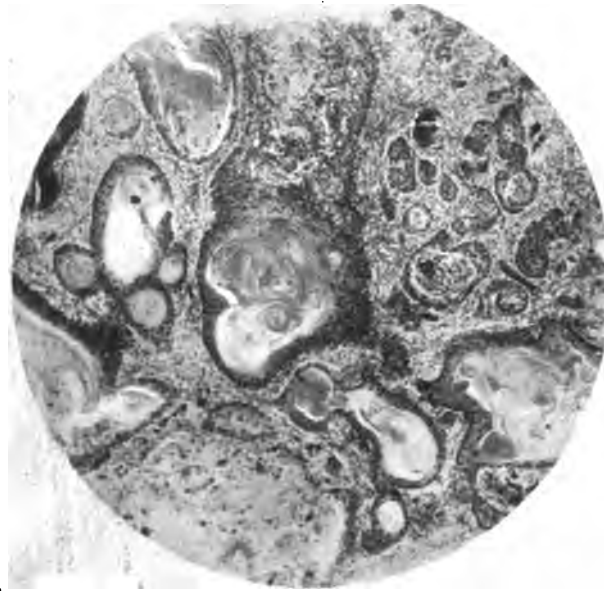


FIG. 2. TYPICAL SQUAMOUS CELL CARCINOMA OF THE JAW, WITH FORMATION OF ABUNDANT EPITHELIAL PEARLS. NO. 23331. $\times 60$

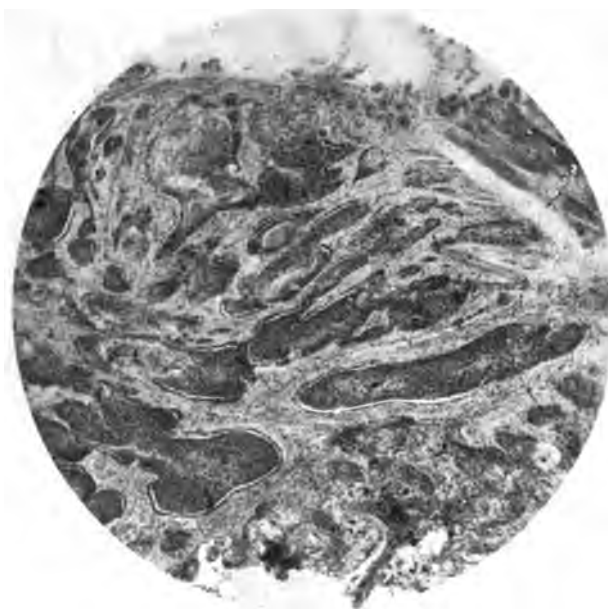


FIG. 3. SQUAMOUS CELL CARCINOMA OF THE JAW INFILTRATING ADJACENT TISSUES EXTENSIVELY. TYPE WITH LITTLE HORNIFICATION. NO. 26238. $\times 60$

all the clinical features of carcinoma has been found at autopsy so extensively necrotized and suppurating that only examination of numerous sections from different parts of the growth has established the diagnosis of carcinoma. It is quite possible that we have lost a few genuine carcinomas through such destructive processes, to say nothing of cases in which the cannibalistic mate has selected the neoplasm for the first course.



FIG. 4. SQUAMOUS CELL CARCINOMA OF SKIN OF CHEST ARISING IN THE
OF AN OLD WOUND, THE SCAR TISSUE BEING ABUNDANT IN THIS FIELD,
AND THE RELATION OF THE CANCER GROWTH TO THE OVER-
LYING SKIN BEING SHOWN. No. 8212. $\times 45$

The microscopic features of the squamous cell carcinoma the skin and mouth of mice differ not at all from those that occur in man. Usually hornification is marked (fig. 2), but, as in man, growths from a similar origin may show little or no tendency to form keratin (fig. 3). Usually the amount of connective tissue formation is not large, except in some cases where the cancer has developed in scar tissue from old wounds (fig. 4). Little help in diagnosis is afforded by mitotic figures,

since these are scanty in even the most typically malignant growths, and commonly cannot be found at all. Presumably they would be much more numerous if the tumors were removed from the living animal.

The infiltrative character of growth is not usually so extensive as we are accustomed to see it in man, for the mice generally succumb to infection from the ulcerated growth while it is



FIG. 5. SQUAMOUS CELL CARCINOMA OF THE HEAD AND FACE INVADING THE SPINAL COLUMN IN THE CERVICAL REGION, COMPRESSING THE SPINAL CORD AND INFILTRATING THE MENINGES
This tumor also invaded the skull. No. 23766. $\times 60$

still small. Infiltration is usually seen best in the tumors that arise about the jaws, for here the bone is often invaded. Bone infiltration was observed in 7 of the 15 tumors that arose about the jaw. We have had one striking case (23766) in which a carcinoma of the skin of the head, beginning at the base of the right ear, infiltrated the skull and cervical vertebral canal, infiltrating the meninges about the cerebellum and compressing the spinal cord in the cervical region (fig. 5). This case resembles

the one described and pictured by Haaland, in which the skull was invaded. In two instances we have seen infiltration of the salivary glands, and in three cases an adjacent lymph node was involved apparently by direct extension.

The absence of lymphatic metastasis is a striking feature of these tumors when compared with corresponding growths in man, which is true of all forms of carcinoma in mice. In only two cases did we find a secondary growth by metastasis into a lymph node from a skin carcinoma (7950, 15232), and it was a very common observation that lymph nodes immediately adjacent to or in contact with these squamous cell growths, were not involved. Possibly serial sections of all our mice would have revealed other instances of metastasis, but the value of the information did not seem commensurate with the labor involved. In only one case did we find visceral metastasis (12627). This mouse had a squamous cell carcinoma arising just dorsal to the rectum, and a typical secondary nodule about 1 mm. in diameter in the lung; it also had a tubular carcinoma of the mammary gland which had produced no metastasis. The infrequency of metastasis in this series of spontaneous squamous cell carcinoma is significant when compared with Yamagiwa's experimental tar tumors in rabbits, since the infrequency of metastasis in his material has been thought by some to speak against their being true malignant tumors. As a matter of fact the metastasis incidence obtained by him, and by Tsutsui with experimental skin tumors in mice, is distinctly higher than that observed in these spontaneous growths.

The sex incidence is strongly in favor of the female (49 to 21) in this series. As most of the growths were on the head and neck we cannot account for this on the basis of erroneous inclusion of squamous carcinomas of the mammary gland with our skin tumors. It differs from our experience with other growths of non-reproductive tissues which have shown approximate equality as to sex incidence. Even more than with other tumors we have found that age is an important factor, these squamous cell carcinomas of the skin being predominatingly in old mice, and as many male mice die early from wounds received in fighting

this may account for the relative preponderance of females in this series.

Beyond the relatively slight extent of infiltration and metastasis these carcinomas present no noteworthy differences from human skin carcinomas. Often the amount of keratin scales piled up on the surface is strikingly great, and in one case the carcinoma arose at the base of definite cutaneous horns (25785). There is often a noteworthy amount of calcification of the necrotic scales. Cyst formation is frequently observed, and benign cutaneous cysts have been observed in several mice.

The basal cell carcinomas all arose on the face, ears, and head (fig. 1), 9 of the 15 being in females. Because these growths are of relatively low malignancy, as in man, they present particular difficulty in diagnosis, and we have set aside as "precancerous" numerous instances of basal cell hyperplasia of marked degree which did not present unqualified proof of malignancy. Quite frequently enormous hyperplasia of the cells about the hair follicles produces tumors of considerable size, and in these may be found areas highly suggestive of malignancy, in that altogether atypical plugs and masses of basal cells are formed, as shown in figure 6. Such growths, which very probably would have shown unqualified malignancy had the mouse lived longer, have not been included among the basal cell carcinomas. In general, basal cell carcinoma in the mouse corresponds entirely to the corresponding growth in man (fig. 7). To quote MacCallum (28), "In spite of the complexity of the downward growing strands all reach to about the same level. Further, it is seen that they are very sharply outlined against the stroma and show little inclination to strew their cells into the irregular crevices of that tissue." We have found the various modifications of basal cell growths commonly described, such as the formation of tubules suggestive of undeveloped hair follicles, hornifying surface areas with typical basal cell growths beneath, and the so-called adenoid epithelioma which seems intermediate between basal and squamous cell growths. Among our keratinizing carcinomas of the mammary gland was one of cells that suggested a basal cell character. No metastases were

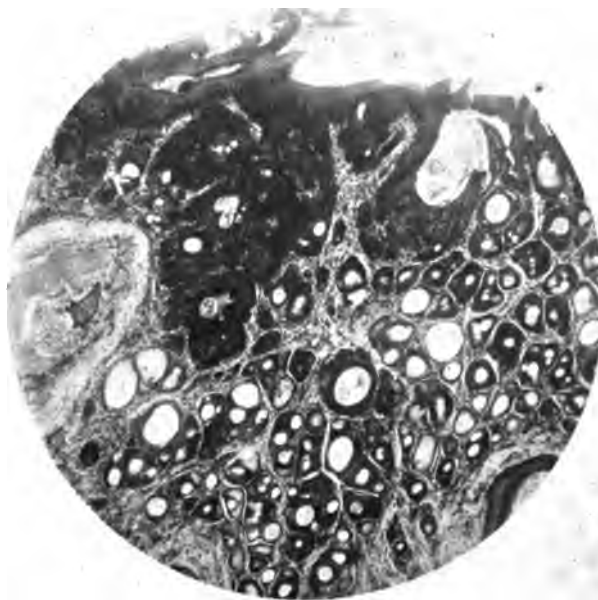


FIG. 6. BASAL CELL HYPERPLASIA OF AN IRRITATED MUZZLE, WITH AREAS OF MORE ATYPICAL GROWTH

There have been numerous such growths, which are probably properly designated as precancerous, but may be actually malignant. No. 16563. $\times 60$.

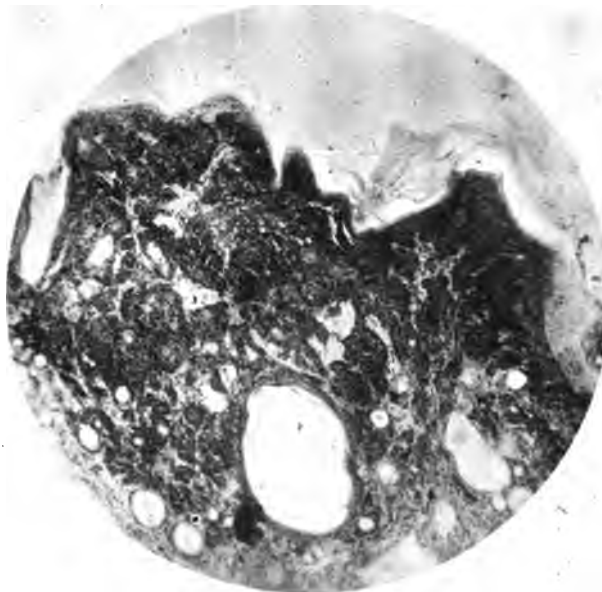


FIG. 7. BASAL CELL CARCINOMA WHICH WAS HIGHLY MALIGNANT, AS IT ENTIRELY REPLACED THE FACE, DESTROYING BOTH EYES, AND FORMING TUMOR MASSES IN THE ORBITS. No. 9932. $\times 90$

observed among these cases, although in one, extensive infiltration destroyed both eyes and produced large tumor masses in the eye sockets. Several of these growths arose at the site of distinct scars from wounds.

Despite the fact that these skin tumor mice were all well advanced in age the co-existence of other tumors is lower than that seen in many other forms of mouse tumor. Perhaps one factor in this is that most of the mice with skin cancer had lived beyond the age at which other tumors occur most frequently. It also seems that the heredity of these mice is responsible to some extent, but a complete analysis of this factor has not yet been made. Of the 85 cases, in which 58 were females and 27 males, but 6 showed a carcinoma of the mammary gland, 7 exhibited lung adenoma (including one that also had a mammary gland carcinoma), one had an adenoma of the ovary and one seemed to have pseudoleukemia. Despite the number of old wounds in these mice no cases of sarcoma were observed, presumably because of either old age or ancestry, but one case of carcinoma arising in the mouth and infiltrating the jawbone, showed such a flattening of the deeper cells that for some time we were in doubt whether or not it was an instance of mixed sarcoma and carcinoma. This case (8560) is described and pictured in our article on sarcoma in mice (29).

SQUAMOUS CELL CARCINOMAS OF THE MAMMARY GLAND

The keratinizing carcinomas of the mammary gland form an interesting group, and properly lie outside the scope of this paper, except for the fact that with not a few of them it is extremely difficult to tell whether we are dealing with a primary mammary gland carcinoma or a skin carcinoma arising over the mammary gland. Three types of these tumors can be distinguished.

1. Carcinomas of the mammary gland which are essentially cylindrical cell carcinomas, forming tubules and alveoli, but some areas of which undergo a transformation into stratified epithelium with the formation of keratin, often in large amounts (fig. 8). These are the "adenocarcinoids" of Murray and

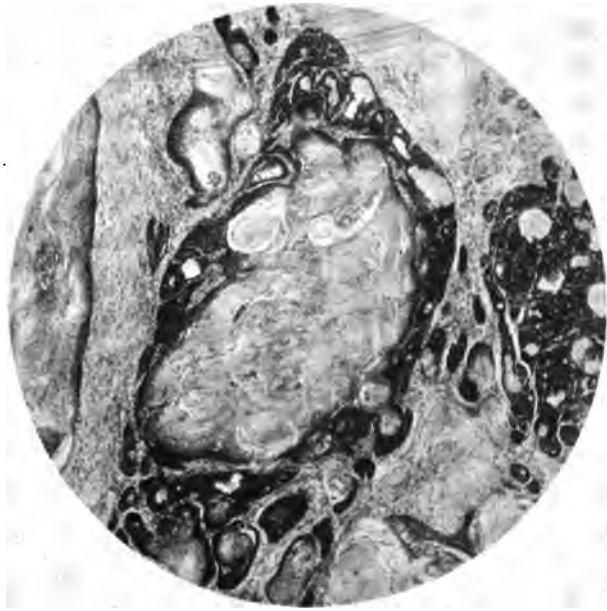


FIG. 8. TUBULAR ALVEOLAR CARCINOMA ("ADENOCANCROID") OF THE MAMMARY GLAND WITH AREAS OF KERATINIZATION

To the left is seen a band of densely keratinized tissue with no evidence of its glandular origin. No. 13336. $\times 60$.

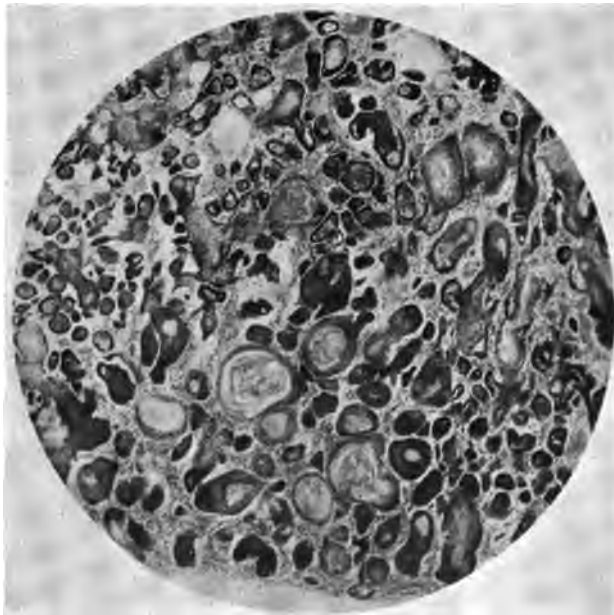


FIG. 9. SUPERFICIAL ADENOCANCROID, APPARENTLY ARISING IN THE DUCTS OF THE MAMMARY GLAND

Shows very little evidence of origin in glandular tissue. This mouse also had three other tubular carcinomas of the mammary gland without keratinization No. 15300 $\times 60$.

Haaland. It is probably of some significance that the keratinization is usually most marked in the portions nearest the cutaneous surface, as if it began in the ducts. The amount of keratinization varies, sometimes appearing in only a few small spots in the tumor tissue, but often extending until little of the original columnar cell type of tissue remains.

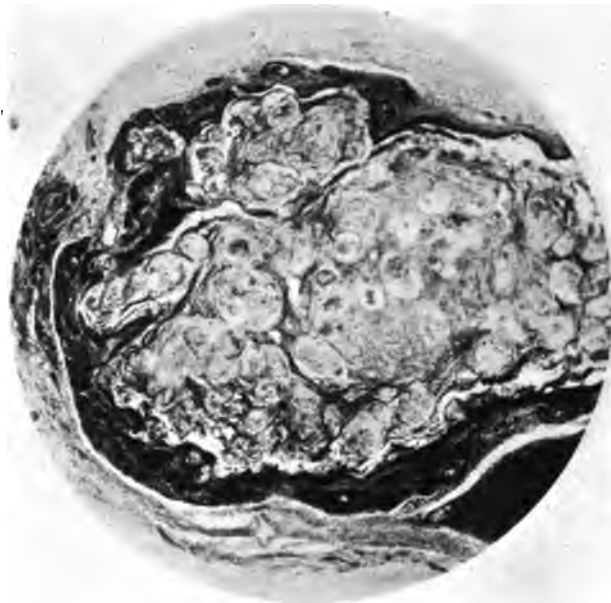


FIG. 10. KERATINIZING CYSTS IN THE MAMMARY GLAND

Such structures are not infrequently found, and may be the precursors of some of the malignant keratinizing carcinomas of the mammary gland. No. 8271. $\times 40$.

2. Squamous-celled keratinizing carcinomas without any evidence whatever of cylindrical cell structure, but arising subcutaneously in the mammary regions (fig. 9). Many of these present no histological evidence that they are derived from the cells of the mammary gland, but they are observed to arise within the gland substance and sometimes are still entirely subcutaneous when the mouse dies. The occasional presence

within the mammary gland of what seem to be simple benign cysts of stratified epithelium with masses of desquamated hornified material (fig. 10) indicates the probable origin of such tumors. When they ulcerate on the surface it may be impossible to differentiate them from primary carcinoma of the skin or nipple, for histologically they differ little if at all from the usual squamous

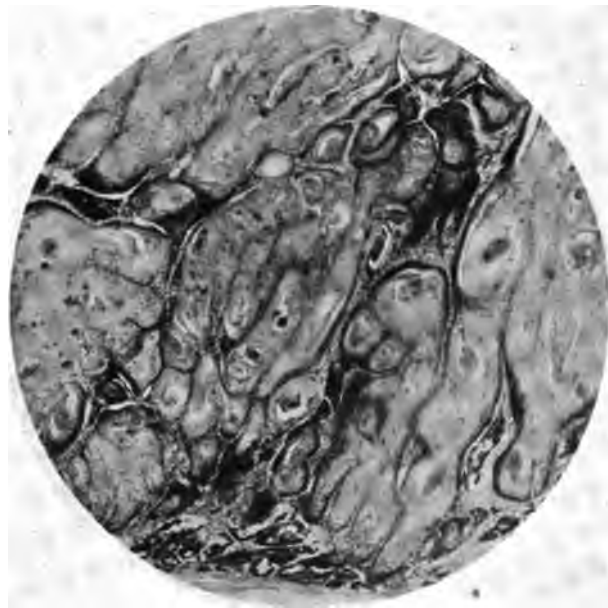


FIG. 11. MOLLUSCOID CARCINOMA OF THE MAMMARY GLAND

This growth, which arose in an inguinal mammary gland, invaded the abdominal wall and protruded into the pelvis, but produced no metastases. A non-keratinizing carcinoma of the mammary gland was also present which produced pulmonary metastases. No. 15622. $\times 60$.

cell carcinoma of the skin. When observed early their origin in the gland beneath the skin is usually the chief ground for recognizing them as mammary gland tumors; nevertheless, the fact that after differentiating, on a histological basis solely, the skin carcinomas from the mammary gland carcinomas we found that nearly all those we had selected as of cutaneous origin had

arisen at the site of old skin wounds, indicates that there are usually recognizable differences.

3. Molluscoid carcinomas of the mammary gland, to adopt Borrel's designation of these striking growths with long, radiating cylinders of stratified epithelium with a large central core of keratinized material (fig. 11). These seem to grow out from the tubules near the nipple, as if they arose from the stratified portion of those ducts. They produce ulceration of the surface about the nipple, which greatly enhances their resemblance to the skin carcinomas.

We have little to add to the statements of Borrel, Murray, Tyzzer, and Haaland concerning these squamous cell growths arising within or about the mammary gland, and the excellent illustrations accompanying Haaland's article make any further illustrations by us unnecessary. A few points may be worth recording. Forty-three of the 56 squamous-celled mammary gland tumors may be best designated as of the adenocarcinoid type, in that they show a greater or less proportion of cylindrical cell structure. Several of these were multiple, as is often the case with primary carcinoma of the mammary gland. In three there were two adenocarcinoids, one mouse had three, and one had five, four arising distinctly in mammary gland tissue while the fifth arose anterior to the urethra and seemed to have originated in the skin, and this showed no glandular elements. Fourteen mice had an adenocarcinoid and also one or more other independent mammary gland carcinomas that showed no keratinization. Of these, one (9365) exhibited also a typical squamous cell carcinoma metastasis in the lung; one mouse with three primary adenocarcinoids (5738) showed a lung metastasis while three mice that had each two mammary gland tumors, one of each type, had pulmonary metastasis of the cylindrical cell type. That is, from 43 cases of adenocarcinoid of the mammary gland, but one metastatic nodule of adenocarcinoid has been seen in the lungs, and none in the lymph nodes or elsewhere. Not a few of these mice had also co-existing tumors other than those of the mammary gland, namely, one with a squamous cell carcinoma of the muzzle (9962), one with a malignant ovarian tumor

(6801), two with uterine fibroids (14370 and 14811); one with a lung adenoma (12098); one with an adenoma of the liver and an adenoma of the lung (9544); one with an adenoma in the lung and a strange growth in a subcutaneous lymph node that might be interpreted as an endothelioma (15622) and one had an intrapelvic sarcoma with metastasis into the liver and also into an ovary which contained in addition a small adenoma (10006).

Two of the adenocarcinoids were examples of the not uncommon mixture of sarcoma and carcinoma in the mammary gland of the mouse (15412 and 6182).

Only four of our tumors were typical of the molluscoid cancers, although more or less of this character was shown by several of the growths diagnosed as adenocarcinoids. One of these four mice had also an adenoma of the ovary (496) and another had a benign lung adenoma (5581). None of these four keratinizing tumors produced metastases.

Nine squamous cell carcinomas that exhibited no glandular structure to identify them as mammary gland tumors, were believed to have this origin because they arose beneath the skin, and in most instances did not ulcerate through at any time. These growths, however, seem to be of a more malignant character than the adenocarcinoids; three of them produced squamous cell metastases. No. 10936, which is remarkable in being the only male with a subcutaneous stratified epithelial growth that seemed to arise in the mammary gland, exhibited squamous cell metastatic growths in the lung, in the mediastinum, and in the chest wall. Another (7950), with three subcutaneous, non-glandular squamous cell carcinomas, showed a metastatic nodule in one lymph node and direct infiltration of a second. No. 13671 with a subcutaneous squamous cell carcinoma and also a cylindrical cell mammary gland carcinoma, had three large metastatic nodules in the lungs all of squamous cell structure. As to multiplicity of tumors in this group there were three independent subcutaneous squamous cell tumors in each of two mice (2239 and 7905), two had a simple cylindrical cell carcinoma of the mammary gland in addition to the squamous cell growth (7526 and 13671), while one had adenomas of the liver and ovary

(14047) and one mouse had leukemia (8236). In this group we have included one squamous cell tumor (24858) that differs from the rest in having the non-keratinized tumor elements suggest strongly the appearance of a basal skin carcinoma, although the growth seemed to arise in the mammary gland.

SQUAMOUS CELL CARCINOMA OF STOMACH AND RECTUM

In a previous paper on primary carcinoma of the stomach in mice (27) we collected records of four reported cases of squamous cell carcinoma arising in the cardiac portion of the stomach to which we added three observed in this laboratory. Since that publication we have observed one more case (24367). This was an old female mouse that for some time before death had been isolated because suffering from tape worm infection. The stomach wall in the cardiac portion was much thickened and small white nodules were scattered over the outer surface. No metastases could be found. In the liver was an encysted cestode, and there was a prolapsed rectum showing much thickening of the wall. Microscopically the nodules in the stomach are of squamous cell carcinoma, apparently still quite early but of typical structure. One small nodule of similar structure is found in the omentum. The rectum shows a marked overgrowth of squamous epithelial plugs on the outer, ulcerated surface of the bowel, and with many of these an infiltrative character is suggested, but apparently this lesion cannot be diagnosed as carcinoma.

Another new case of carcinoma of the stomach (25911) is more difficult to classify. A female *Peromyscus* mouse, nearly four and a half years old, which had been isolated all her life and never bred, refused food for several days before death. At autopsy there was found a marked thickening of the entire stomach wall, particularly at the cardiac end where it seemed nearly solid from wall to wall. A thick white nodular outgrowth 6 by 4 by 4 mm. of similar tissue bound together the stomach, liver, and esophagus, while another nodule 8 by 4 by 4 mm. bound together the pancreas and intestinal mesentery posterior

to the stomach. No other metastases were found. Microscopically this growth is composed of groups of strands of infiltrating epithelial cells, which are not hornified, neither do they form tubules. They grow profusely throughout all coats of the stomach, sometimes forming sheaths about the tubules of the cardiac portion. The nodules outside the stomach are secondary growths of identically the same structure in lymph nodes. It is not possible to be sure whether this carcinoma is derived from the columnar or the stratified epithelial portions of the stomach. While the gross appearances suggested an origin in the cardia, which is the site of all but one of the recorded cases of gastric carcinoma in mice, yet all of these were frankly hornifying squamous cell carcinomas.

The full details of the three other cases of squamous cell carcinoma occurring in this series are published with illustrations in our previous communication (27) and need not be repeated here. To recapitulate them briefly they were as follows:

No. 5802, male two years old. Squamous cell carcinoma of pyloric portion of the stomach, measuring 15 by 15 by 12 mm. A secondary growth 11 by 12 mm. in the mesentery. No other metastases. Some infiltration of the pancreas.

No. 7851. Male, twenty-five months old. Cardiac portion of the stomach is ulcerated and thickened, forming a mass 16 by 12 by 8 mm. No metastasis. Structure, typical squamous carcinoma, infiltrating all coats of the stomach, and invading the adhesions between the stomach and liver.

No. 16440. Female, aged twenty-seven months. At the junction of the cardiac and pyloric portions a thickened mass from 3 to 15 mm. wide surrounds the stomach. Nodules were found in the omentum and mesentery, metastases in lymph nodes. Structure, typical squamous cell carcinoma with some hornification.

In the same paper was published with illustrations the report of a case of squamous cell carcinoma arising in the metaplastic epithelium covering a prolapsed rectum (8345). This was a male mouse, which had had a prolapsed rectum for six months before its death. The growth was not extensive but seemed to be

typically squamous cell carcinoma arising in metaplastic epithelium. Since then we have observed a second similar case.

No. 20052. Female, had a prolapsed rectum for four months before death, which was from hypertrophy of the heart and pulmonary edema. The surface of the prolapse became much ulcerated and after a time showed what seemed to be a proliferation of epithelium. The regional lymph nodes were somewhat enlarged. Microscopically the external surface of the prolapsed bowel shows, in addition to considerable ulceration, areas of definite squamous cell carcinoma, with strands of cancer cells infiltrating through a thick layer of granulation tissue down to but not into the muscularis. Apparently this has arisen from what was part of the cutaneous surface of the anus, and not from metaplastic mucosa as in the previous case. As a possible factor in the inciting irritation, a small piece of wood was found imbedded in the bowel wall, surrounded by granulation tissue and cancer cells.

CARCINOMA OF THE VULVA AND VAGINA

No cases of squamous cell carcinoma of the uterus have as yet been described in mice, so far as we can learn. Although we have found a few fibromyomas and sarcomas of the uterus, we have met with but one epithelial neoplasm, which was an adenocarcinoma.

Erdheim (23) and Haaland (20) each have described a recurrent verrucous growth of the vulva, and the latter a mixed squamous and sebaceous cell carcinoma of the vulva. Two cases of squamous carcinoma of the vulva have been observed in this series and are included among the 70 squamous cell skin carcinomas. (1) No. 7950. This occurred as a condylomatous growth arising distinctly in the vulva of an old mouse, infiltrating the subcutaneous tissues, and was an unquestionably malignant squamous cell growth microscopically. There was in addition metastatic growth in two lymph nodes in the groin. (2) No. 18928. A growth of a warty character and ulcerating slightly developed on the vulva some time before the death

of the mouse, spreading about the rectum before death. At autopsy there was found in addition an enlarged lymph node attached to the right ureter. Microscopically the external growth is a typical squamous cell carcinoma with much hornification, and not very marked tendency to infiltration. The nodule attached to the ureter was a lymph node containing a cyst lined with squamous epithelium, or, to describe it better, an epithelial cyst covered with a thin layer of lymphoid tissue. The epithelial wall of the cyst is thin, shows no evidence of infiltrative or other malignant character, and hence does not at all resemble a metastatic growth, but it is difficult to explain the presence of such a structure in this location on any other basis.

In addition to these two carcinomas we have observed an excellent case of carcinoma of the vagina.

Carcinoma of vagina (no. 22582). This mouse was found, nearly a month before its death, with a 3 mm. pink nodule protruding from the vagina. At the time of its death from pneumonia it presented an ulcerating mass 15 by 12 by 10 mm., about one-half of which protruded from the vulva. It seemed to arise from the vaginal wall, the uterus and bladder not being involved, but it ulcerated into the rectum and was much infected and ulcerated. No enlarged glands or other evidences of metastasis could be found. Microscopically the growth is composed mostly of loose masses of keratinized scales exfoliated from the underlying growth, which infiltrates the vaginal wall as a typical squamous cell carcinoma.

KERATINIZING TUMORS OF THE LUNG

As mentioned in our review of the literature, Haaland has described one case of primary keratinizing growth in the lung, and Tyzzer says that he has had "several cases." The influence of heredity on the incidence of tumors of special types is suggested by the fact that of several hundred cases of primary lung tumors in the Slye stock, but one has been definitely found to show keratinization.

No. 13314. A male, age twenty months, which showed no other autopsy findings of interest, had the lower lobe of the left

lung nearly replaced by a yellowish mass, which was distinguished from the ordinary papillary adenoma of the lung chiefly by its color. This made it resemble an abscess, but it was much too hard to be an abscess, and there was no pleural exudate or adhesion. Microscopically the tumor consists chiefly of a mass of hornified scales heaped up in waving, concentric layers. Only at the very periphery are living cells found. Here is a narrow growing border of stratified epithelial cells, differing in no essentials from that seen in epitheliomas of the skin. There is no marked tendency to infiltration, the growth apparently progressing by expansion, but the presence of occasional mitotic figures is noted. About the growth there is much round and spindle cell proliferation and numerous foreign body giant cells are found. Our specimen differs from the one illustrated by Tyzzer in having a smaller proportion of living cells. We are not certain whether this growth represents a true neoplasm or a progressive metaplasia due to some persisting chronic inflammatory condition, but the former seems more probable.

Two other mice have shown somewhat related pulmonary conditions.

No. 10561. This mouse, which had also a carcinoma of the skin, had a benign adenomatous growth in the lung which showed some tendency to stratification, but without keratinization.

No. 25136. A male mouse had in the right upper lobe of the lung a mass 14 by 12 by 10 mm. resembling in appearance a malignant tumor of the lung. Microscopically this tissue resembles much more closely an unresolved organizing and necrotizing pneumonia, in which are two irregular, independent areas composed of masses of keratinized scales, with a slender border of flattened epithelial cells. It is quite impossible to decide whether this is a true tumor, or whether it is the cause or the result of the pneumonic condition, although the epithelial growth has the appearance of being much older than the pneumonic process.

TUMORS OF THE MEIBOMIAN GLAND

Two mice have presented growths arising in the eyelids, which, according to their structure, seem to be adenomas arising in the Meibomian glands.

No. 18099. A small, slowly growing mass developed beneath the left eye of a female mouse; from the eye exuded a small amount of thick white exudate. The growth had reached a diameter of 10 mm. when the mouse died from an acute lung infection, and showed no evidence of infiltration or ulceration. Microscopically the growth is composed of papillary structures covered with many layers of epithelial cells. It differs from the normal Meibomian gland in the exaggeration and lawless arrangement of the structures, and the greatly increased number of epithelial cells covering the stroma. The diagnosis of benign adenoma of the Meibomian gland seems justified, especially in view of the size of the growth and the findings in the next case.

No. 27929. An old female mouse, which died of chronic nephritis, had a soft mass, 6 mm. in diameter, beneath the left eye. The gross appearance suggested an epithelioma. Microscopically this tumor is quite the same as the one described immediately above, except for the important fact that it infiltrates down to the bone of the orbit, thus indicating that it is a malignant infiltrating adenocarcinoma.

SEBACEOUS GLAND ADENOCARCINOMA

Such tumors have been described in mice by Murray, Tyzzer, and Haaland, the last two having transplanted them successfully. We have found one case of sebaceous adenocarcinoma of the preputial gland, which closely resembles the growth described and illustrated by Haaland (20).

No. 19895. Beginning at the base of the penis is a mass 30 by 25 by 25 mm. extending well into the inguinal region. The penis was completely imbedded in the tumor, the older portions of which were softened, but about the periphery were hard nodules of newer growth. The testicles and epididymis were

not involved. Death resulted from chronic nephritis. There were no metastases. Microscopically this tumor (fig. 12) reproduces closely the normal structure of the preputial gland, but not infrequent infiltration of the stroma by strands of epithelial cells corroborates the gross evidences of malignancy. It corresponds perfectly to the illustration given by Haaland, who also

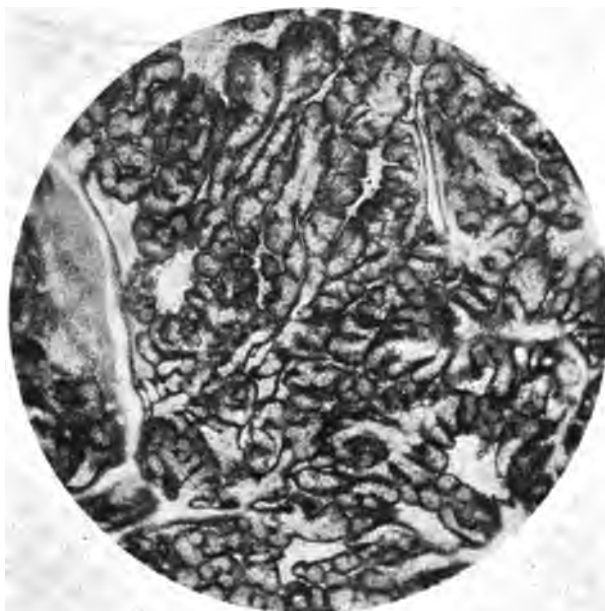


FIG. 12. SEBACEOUS ADENO-CARCINOMA OF PREPUTIAL GLAND

This was a large tumor with distinctly malignant characteristics, infiltrating the adjacent tissues, despite its close resemblance to the normal structure of the gland from which it arose. No. 10895. $\times 60$.

comments on the close resemblance to the normal gland in spite of the definitely malignant character.

SUMMARY

Among 28,000 mice dying natural deaths at all ages, and carefully autopsied, have been observed 153 growths of stratified and squamous epithelium that correspond by the usual

standards to true neoplasms, excluding a considerable number of epithelial growths which lack positive conclusive evidence of neoplastic character, although possibly some of these are also early carcinomas. Seventy-one are examples of squamous cell carcinoma of the skin or mouth. They differ from the human skin carcinoma chiefly in a low incidence of metastasis. Fifteen others are of basal cell character, arose always about the head, and produced no metastases. In both these groups the incidence is higher in the females than in the males. Trauma and chronic irritation seem to play an important part in the production of skin carcinoma in mice, most of our cases occurring about the head and face, often recognizably at the site of wounds, and nearly all the skin carcinomas of the trunk arose in old scars. Skin cancers occur at a greater average age than other tumors in mice.

Fifty-six examples of squamous cell keratinizing growths arising in the mammary gland were observed, predominatingly adenocarcinomas with localized areas of keratinization. These also seldom produce squamous cell metastases.

Other tumors in this group were: Four squamous cell carcinomas of the stomach, two arising in the prolapsed rectum, two in the vulva, one keratinizing tumor of the lung, one sebaceous adenocarcinoma of the preputial gland, and, as hitherto undescribed mouse tumors, one squamous carcinoma of the vagina and two adenomas of the Meibomian glands, one of these being infiltrative and apparently malignant.

The literature of the comparative pathology of squamous cell carcinoma in animals is reviewed, and it is worthy of comment that as yet no cases of squamous cell carcinoma of the uterus, bladder, or esophagus seem to have been described in mice.

REFERENCES

- (1) STICKER: Arch. f. klin. Chir., 1902, lxx, 616, 1023.
- (2) TEUTSCHLAENDER: Ztschr. f. Krebsforsch., 1920, xvii, 285.
- (3) JOEST AND ERNESTI: Ztschr. f. Krebsforsch., 1915, xv, 1.
- (4) FOX: Jour. Path. and Bacteriol., 1912, xvii, 217.
- (5) BOYNTON: Festschrift for Victor C. Vaughan, 1903, p. 535.
- (6) URRÁ: Rev. de med. y cirug. práct., Madrid, 1918, cxviii, 321.

- wild mice
- (7) TEUTSCHLAENDER: Ztschr. f. Krebsforsch., 1920, xvii, 350.
 - (8) FIEBIGER: Ztschr. f. Krebsforsch., 1909, vii, 165.
 - (9) ROFFO: Rev. d. Inst. Bacteriol., Buenos Aires, 1918, 1, 333.
 - (10) BASHFORD: Third Sci. Rep., Imperial Cancer Research Fund, London, 1908, p. 41.
 - (11) LOEB: Arch. f. klin. Chir., 1903, lxx, 845.
 - (12) WOOLLEY AND WHEBBY: Jour. Med. Res., 1911, xxv, 205.
 - (13) HANAU: Fortschr. d. Med., 1889, vii, 321.
 - (14) ROFFO: Rev. d. Inst. Bacteriol., Buenos Aires, 1918, i, 155.
 - (15) LIPSCHÜTZ: Wien. klin. Wchnschr., 1920, xxxiii, 426.
 - (16) BULLOCK AND ROHDENBURG: Jour. Cancer Res., 1917, ii, 39.
 - (17) MORRIS: Jour. Cancer Res., 1920, v, 147.
 - (18) HAALAND: Ann. de l'Inst. Pasteur, 1905, xix, 165.
 - (19) MURRAY: Third Sci. Rep., Imperial Cancer Research Fund, London, 1908, p. 69.
 - (20) HAALAND: Fourth Sci. Rep., Imperial Cancer Research Fund, London, 1911, p. 1.
 - (21) TEUTSCHLAENDER: Ztschr. f. Krebsforsch., 1920, xvii, 300.
 - (22) PICK AND POLL: Berl. klin. Wchnschr., 1903, xl, 518.
 - (23) ERDHEIM: Ztschr. f. Krebsforsch., 1906, iv, 33.
 - (24) TSUTSUI: Gann, 1918, xii, 17.
 - (25) TYZZER: Jour. Med. Res., 1909, xxi, 479.
 - (26) JOBLING: Rockefeller Inst. Monographs, 1910, i, 81.
 - (27) SLYE, HOLMES AND WELLS: Jour. Cancer Res., 1917, ii, 401.
 - (28) MACCALLUM: Text-book of Pathology, Philadelphia, 1920, p. 1032.
 - (29) SLYE, HOLMES, AND WELLS: Jour. Cancer Res., 1917, ii, 1. Fig. 3.
 - (30) WOGLOM: Jour. Cancer Res., 1917, ii, 471.
 - (31) FIEBIGER: Jour. Cancer Res., 1919, iv, 367.

PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH

THIRTEENTH ANNUAL MEETING

Held in New York, April 1, 1920

1. REPORT OF THE COUNCIL

The meeting of the Council was held at Dr. Wood's apartment in New York City, on the evening of Wednesday, March 31, 1920.

The following members were present: Dr. H. Gideon Wells, president; Dr. Robert B. Greenough, vice-president; Dr. Francis C. Wood, and Dr. Willy Meyer, and, by invitation Dr. Frederick Prime. Absent, Dr. E. R. LeCount, Dr. James B. Murphy, and Dr. William H. Woglom.

The report of the treasurer for the year showed a balance on hand of \$532.27.

The status of the JOURNAL OF CANCER RESEARCH was discussed, and a statement from the publishers, showing a total deficit of \$110.80 on the four volumes so far published, was read. After some discussion of the proposal, made by the publishers, that the price of the JOURNAL be increased to \$6.00 for subscribers not members of the Association, it was voted that no change be made until after the completion of the publication of Volume V.

In order to increase the membership of the Society it was decided that the president should send out a letter to certain men whose names were presented as possible candidates for active and associate membership.

The name of the following applicant came before the Council for election to the Association:

Dr. Michael Levine, Montefiore Home, New York City.

Dr. Wood moved and Dr. Wells seconded the motion that he be elected to membership in the Association.

The resignations of the following members were accepted:

Dr. J. George Adami, Dr. Martha Tracy.

The deaths of Dr. M. J. Herzog and Dr. James Douglas were noted.

Dr. Wood moved that Dr. James Ewing be elected councillor succeeding the retiring councillor. Dr. Greenough seconded this motion.

The following officers were elected by the Council to serve for the ensuing year. Dr. Robert B. Greenough, president; Dr. James B. Murphy, vice-president; Dr. William H. Woglom, secretary and treasurer (re-elected).

The present Council, therefore, with the years of retirement, is as follows:

Dr. E. R. LeCount, 1921	Dr. William H. Woglom, 1924
Dr. F. C. Wood, 1922	Dr. Robert B. Greenough, 1925
Dr. James B. Murphy, 1923	Dr. Willy Meyer, 1926
Dr. James Ewing, 1927	

The Council continued in office the present Editorial Board, which is composed as follows:

Dr. William H. Woglom	Dr. Leo Loeb
Columbia University	Washington University
Dr. Frederick Prime	Dr. Ernest E. Tysser
Columbia University	Harvard University
Dr. Joseph C. Bloodgood	Dr. H. Gideon Wells
Johns Hopkins University	University of Chicago
Dr. James Ewing	
Cornell University	

2. A FEW LATE RESULTS AFTER THE RADICAL OPERATION FOR CANCER OF THE BREAST

Dr. Willy Meyer (New York):

SUMMARY

The data given are from the author's private cases which he has followed for the last twenty-six years.

Two radical operations for cancer of the breast have been before the profession since the fall of 1894. Their principal point of difference is the direction in which the surgeon proceeds. One method starts from the chest and works toward the axilla, leaving the clavicular portion of the pectoralis major behind; it requires entering the space between the pectoralis major and minor muscles, the latter usually being divided and then sutured. This method necessarily involves loss of blood. The other method, practised by the author since September 12, 1892, starts from the axilla and works toward the sternum. The tendons of the pectoralis major and minor are divided in the early stage of the operation, necessitating complete excision of both muscles. Blood- and lymph-vessels are primarily divided within the axilla. The lymph-nodes and axillary fat are lifted out in connection with the tumor, before the cancerous breast itself is handled. The entire mass is removed without entering what he calls the "infected area." Hemorrhage is reduced to a minimum.

The final results of the operation from the sternum toward the shoulder, as reported, have been good. Still, small cancerous nodes have been found repeatedly between the pectoralis major and minor muscle, and where cancerous lymphatic nodes have developed, there must be present suspicious lymphatic vessels.

Previous to 1894, excision of the breast for carcinoma was done in two stages, but at the same sitting, first, the removal of the breast with axillary contents; then, the excision of the pectoralis major muscle. This arrangement forced the surgeon to enter widely "the infected area" and caused an unnecessarily great loss of blood. Meyer did not see a single lasting cure after this method of advance. The radical operation changed the results with one stroke, as is shown by a comparison of the author's statistics before and after September, 1894. The first two patients subjected to the modern radical operation were completely cured, and case 4 of this series had for many years enjoyed freedom from cancerous recurrence when she died of old age.

The following patients in the series are alive and well today, from twelve to twenty-five and a half years after operation:

Case 1. Operation in September, 1894; patient thirty-eight years of age (now sixty-four). This was the first case operated upon by the method outlined above. The patient is alive and well today, twenty-five and a half years after operation.

Case 2. Operation in 1895; patient forty-eight years of age (now seventy-three). This woman is alive and well today, twenty-five years after operation.

Case 3. Operation in July, 1902; patient thirty-three years of age (now fifty-one). The patient is alive and well today, eighteen years after operation.

Case 4. Operation in December, 1903; patient thirty-six years of age (now fifty-three). The patient is perfectly healthy and free from recurrence today, seventeen years after operation.

All these patients have full use of the arm and are able to assume the posture of the "Statue of Liberty."

Case 5. Operation in July, 1908; patient thirty-five years of age (now forty-seven). She is perfectly healthy today, twelve years after operation.

Case 6. This patient was operated on in September, 1917, and the case is added merely to show the present line of incision with Handley's addition down to a point midway between the umbilicus and the xyphoid process, for the excision of the fascia covering the upper portion of the recti muscles, in conjunction with the other mass. This addition is recommended because it makes the operation more radical and usually makes possible closure of the wound, without grafting.

Five other patients remained free from recurrence for 4, 6, 8 (2 cases), and 16 years, respectively, and then died of other diseases.

Another patient, a pronounced diabetic at the time of the operation, was well for six years after it, when she succumbed to diabetes, without having developed any signs of a recurrence of the cancer.

Still another patient, operated on for cancer of the right breast in March, 1899, returned in December, 1900, with a carcinoma of the left breast, which was then also extirpated. She was well and free from recurrence when last heard from, in the spring of 1907, six and a quarter years after the second operation.

A few days ago, the author saw a patient now almost eighty years old and in perfect health, who had been operated upon by him for a scirrhous of the breast at the age of seventy-three (seven years ago).

These results, he believes, prove the efficiency of the method; they prove that the *radical operation for cancer of the breast can cure patients*

thus afflicted. That not all the patients are saved is due (1) to the stage of the disease in which they reach the surgeon, and (2) to the virulence of the agent that produces the carcinoma.

The importance of the follow-up system was discussed in connection with the collection of statistics on the late results of operation.

Paget's disease (epithelioma of the nipple)

Paget's disease is the most malignant of known forms of cancers of the breast. If ever early and radical operation is imperative, it is in these cases, as will be seen from the following three observations which were made during the last two years.

Case 1. Female, thirty years old, mother of five children, had been in the hands of a quack and had been treated by caustics. When seen in January, 1918, the disease in the breast and axilla had far advanced. After the radical operation the other breast soon became affected, and one year later, also was excised. Then, not long after, the disease became disseminated, and the patient died from cancer en cuirasse.

Case 2. Female, thirty-eight years old, had been in the hands of one of our best x-ray specialists in the city. One and one-half years after cure of the disease of the nipple by radium treatment, there was a local recurrence and a very extensive cancer of the breast, with infected nodes in the axilla and along the subclavian vein. Radical operation was done in December, 1918, followed by renewed x-ray and radium treatment. She now has developed intrathoracic metastases.

Case 3. Male, forty-seven years old, had been in the hands of an experienced surgeon who had extirpated the breast only, without the axillary nodes, evidently because none could be found at that time. One and three-quarter years later, the patient presented a far advanced carcinoma. The radical operation then performed could not save him; and he died from general metastases eight months afterward.

In operating upon mammary carcinoma, Meyer makes it a point to circumsise the skin widely at the base of the breast. He prepares two ample flaps and enfolds them extensively, then divides the fasciae at the base of the two flaps, and extirpates them together with the mass. Involvement of the supraclavicular nodes does not, in his opinion, present a contraindication to operation; on the contrary, he considers it the surgeon's duty to operate when these nodes are infected. Halsted, as well as the late Rodman, have observed patients who remained well for a number of years after the extirpation of these nodes.

In none of the cases presented were the supraclavicular nodes found infected at the time of operation, and hence they were not removed. Meyer has not operated on a single case in which there were no infiltrated axillary nodes.

Meyer believes that statistics regarding the results of the radical operation for cancer of the breast are worthless. They do not prove anything. What *does* determine the fate of the patients is the so-called virulence of the disease. One and the same surgeon may do an equally radical operation in two seemingly early or apparently equally far advanced cases, in one of which the patient may remain well and

free from recurrence for, say, twenty-five years, while in the other a regional recurrence and metastases may develop within a few months. All that can be said is that cancer, being a local disease in the beginning, may be cured by a radical operation, if this is done at an early stage.

DISCUSSION

Dr. Robert B. Greenough (Boston); I feel we must acknowledge that surgery of the breast owes much to Dr. Meyer for the work he has been doing, and I am entirely in accord with his reasons for operating in the way which he recommends, namely, beginning the operation in the axilla, and removing the axillary contents, the whole of the breast, both of the pectoral muscles, and the fascia of the chest wall, all in one piece. I feel this hardly needs argument, because it is an accepted principle in operating for the radical cure of cancer in any situation where the anatomical conditions make it possible. I personally have had more experience with the transverse axillary incision than with the incision used by Dr. Meyer, but I think the choice of the incision is a very small matter. It is far more important what is done beneath the skin, and I am entirely in accord with Dr. Meyer in that respect.

In regard to the presentation of statistics in cases operated on for the cure of cancer, some fourteen years ago I had the opportunity of reporting, at the meeting of the American Surgical Association, the results of operations for carcinoma of the breast at the Massachusetts General Hospital, and at the same meeting other papers on the end results of cancer at many different institutions were also presented. Dr. Halsted opened the meeting, and the very first words of his paper were of great significance. He said: "It is especially true of breast cancer that the surgeon interested in furnishing the best statistics may in perfectly honorable ways provide them."¹ I believe that if we are going to attempt a comparison of different methods of operating we must have some definite standard which we accept. I know no better standard than to take all the cases that come into a general hospital under the diagnosis of the disease in question and to record them during the period under observation, with a statement as to what percentage of that number was considered suitable for the attempt at radical cure, and what results were obtained. I am sorry that the idea has not taken hold better in the general reporting of cases. I was looking at a report of cases of cancer of the lip recently published, and in that report all the cases in which an attempt to trace the patient after operation by letter had been unsuccessful, were wiped out of the record. It has been our experience in Boston that the patients from whom one cannot hear are usually those that have actually left this world for another, and I fully believe that patients untraced should be counted as failures. I believe also, as Dr. Meyer stated so emphati-

¹ Halsted, W. S.: The Results of Radical Operations for the Cure of Carcinoma of the Breast, *Ann. Surg.*, 1907, xlv, 1.

cally, that one of the great functions of the social service departments of these hospitals should be the tracing of end results. At the Huntington Hospital we have an excellent social service worker who has established a standard in regard to these things, and we do not consider a record closed when the patient has been discharged until we have definite information of the end result. We keep them as live cases until we know that they are dead.

3. PRIMARY SPONTANEOUS TUMORS OF THE OVARY IN MICE—STUDIES IN THE INCIDENCE AND INHERITABILITY OF SPONTANEOUS TUMORS IN MICE. FOURTEENTH REPORT

Miss Maud Slye, Miss Harriet F. Holmes, and Dr. H. Gideon Wells (Chicago):

SUMMARY

Among 22,000 mice of the Slye stock dying natural deaths at all ages were 44 with spontaneous primary ovarian tumors, not including simple ovarian cysts. Of these, 38 had simple benign solid papillary adenomas, only occasionally with slight cyst formation; 1 showed a typical papillary cystoma, and 1 a typical solid teratoma containing a great diversity of tissue elements. A second case of this type has since been found in the first 25,000. Of the 38 cases of solid papillary adenomas, 19, or 50 per cent were bilateral, so that there were 57 tumors of this class. There were 4 unquestionable primary malignant tumors of the ovary, all showing the "mesothelioma" type of growth characteristic of malignant tumors derived from the sex glands; one of these produced perirenal metastases. One other tumor of the same type was primary in either the ovary or the adrenal. Two round-cell sarcomas were found, arising either from the ovary or from some other organ, while 2 other sarcomas had produced secondary growths in the ovary. Of the 44 mice with primary ovarian tumors, 26 had tumors in other parts of the body.

In the literature were found reports of eight other cases of benign tumors arising in the ovaries of mice, all exhibiting the same characteristics as the tumors described in this paper.

DISCUSSION

Dr. F. C. Wood (New York): Statistics of this sort are of vital importance in all experimental work in cancer, since they show that if animals are kept to a sufficient old age there is a very large incidence of tumors of all varieties. I think that we who experiment with animal tumors are still supposed by the clinical fraternity in medicine to be working with something which is entirely different from and not in the least comparable to human tumors. But as observations on animals are being extended over larger series of mice, and to other species, as white rats, dogs, guinea-pigs, etc., we are finding instead that tumors abso-

lutely comparable in morphology and biological qualities occur in many of our domesticated animals. This is interesting as furnishing an argument against the current opinion that tumors are a disease of civilization, and primarily of the educated classes, and not a disease which is widespread and generalized through all groups of society. The statistics of the Metropolitan Life Insurance Company, which show a greater incidence of cancer among the laboring classes, point in the same direction, that is, to the fact that there is no immune class. Obviously, in the manual laboring classes the conditions of irritation and of infection (for instance, syphilis, which, while not causing cancer, facilitates its occurrence) result in a larger proportion of tumors in that group, and as we study mice we find that larger numbers of tumors spontaneously appear in these animals. I do not doubt that the same would be found to be true of wild mice if we could keep larger numbers to old age.

Dr. James W. Jobling (New York): I should like to ask Dr. Wells if these ovarian tumors were observed more frequently among the "tumor strains" of mice described by Miss Slye.

Dr. Wells: In reply to Dr. Jobling's question: Miss Slye had hoped to be able to discuss that feature, as, of course, the study of heredity is entirely her part of the work; but on account of illness she was not able to get the material together. We have had strains for twenty-five or thirty generations producing enormous numbers of mice with no tumors. It is perfectly safe to say that mice of certain strains are more likely to have tumors of the ovary than are those of other strains. It is a familiar fact that in the strain of mouse tumor that is used in most laboratories in America, that derived from the Abbie Lathrop stock in Granby, Massachusetts, cancer of the mammary gland is the usual type. Very few cases of tumors of any other tissue have been described in this stock; but Haaland's reports indicate that in the mice in the Imperial Cancer Research Fund in London, tumors of other organs are quite common. I may recall Miss Slye's observations, previously reported, that with tumors of specific organs a very definite relationship to heredity is shown. She reported at one time twenty-eight cases of primary tumor of the testicle, all, with one exception, arising in mice of one definite strain. The exception was in a mouse which had been bitten on the testicle, and which belonged to a strain in which sarcoma was very common. The most striking series which Miss Slye has had was the liver tumor. You may recall that the literature of mouse tumors had previously shown but one case of primary tumor of the liver; since then one or two others have been described. Now Miss Slye has bred, from a mouse with primary tumor of the liver, a strain in which there have developed one hundred cases of primary growth in this organ. While there are no figures on the ovarian tumors, we know that most of them arise in certain strains, though whether strictly within the strains, as in the case of the testicle and liver tumors, we are not yet prepared to say. But in such experi-

ments as these, certain difficulties must be faced. Thus Tyzzer and Haaland have described squamous-cell neoplasms of the lung, a type of which we have seen only one or two examples. Hence minute differences must exist, making one strain liable to one variety of tumor in any given organ, and another liable to a type slightly different.

Dr. C. C. Little (Cold Spring Harbor, N. Y.): How would you explain, on the basis of a single Mendelian factor, the occurrence of these clearly demarcated strains which show specialized types of tumors in particular organs or groups of organs?

Dr. Wells: I am not prepared to discuss that aspect of the work at all; that I leave entirely to Miss Slye. I do not consider myself competent to discuss the matter of genetics in these problems.

Dr. Little: I would like to point out that the result outlined by Dr. Wells is very interesting in support of the theory that the hereditary nature or susceptibility to the occurrence of spontaneous tumors depends on *more than one hereditary factor*. This is evidenced by the clear ability of a particular family to localize its susceptibility to tumor in a particular organ, and is further evidenced by the ability of other families to combine tumors of different organs. Such a result is particularly characteristic of the action of many factors in heredity, rather than of only one. I mention the fact because of the possibility that too much emphasis may be placed on a single factor as the cause of all cancer. The specificity of tissues is so great that it would be surprising if such evidence as that given by Dr. Wells were not found when so careful a study was made.

Dr. William C. Stone (New York): I am very much interested in this presentation because of my own study of ovarian tumors in the human subject. In the first place, as to diagnosis: Dr. Wells spoke of a certain number of these tumors as possibly endotheliomata. Similarly in the human cases, in going over the literature of the so-called Krukenberg tumors, one finds numerous instances in which the picture has been interpreted by many as an endothelioma, by others as a sarcoma, and by still others as a carcinoma. Then, as regards the observation of the occurrence of the tumors in both ovaries, and the difficulty of explaining this in human patients, also, in a large number of instances the ovarian tumors are of secondary nature, and the primary site is elsewhere; but there are numerous cases in which no primary site can be found, and the incidence of the tumor in both ovaries is unexplained. There is one difference, however, if I understand Dr. Wells correctly, between human and mouse tumors. In the majority of cases the so-called Krukenberg tumors in women seem to be secondary in the ovaries. If one considers carefully the descriptions of those reported as primary, one is unable to exclude the possibility that in the majority there was a primary site elsewhere. We found it most frequently

in the stomach, gall-bladder, appendix, or some other part of the intestinal tract.

Dr. Wells: Of course, in these cases the histology is not that of the typical Krukenberg tumor. The tumors are bilateral, but they do not resemble the Krukenberg tumor. They do show characteristics which I am quite sure would be cause for a diagnosis of endothelioma or of sarcoma; in fact, before I had studied these tumors carefully I labelled a good many of them endotheliomata of the ovary. It may be interesting to know that every once in a while we see in bovines bilateral tumors of the ovary; some of those I have seen from the stockyards in Chicago are histologically similar to the Krukenberg tumor although nothing is found to indicate a primary tumor elsewhere in the cattle. In mice there have been reported only five or six cases of primary abdominal tumors which could give rise to Krukenberg tumors; so that here we have tumors which are bilateral, and which are distinctly not secondary tumors. Why they should be bilateral in 50 per cent of the cases, and why bilateral tumors are so much more likely to occur in the ovaries I am unable to explain.

4. THE RÔLE OF NEOPLASIA IN PARASITIC DISEASES OF PLANTS.

Dr. Isaac Levin and Dr. Michael Levine (New York):

Previous investigation by the authors on the crown gall have demonstrated that while this condition frequently acts in a manner analogous to animal cancer, the cellular proliferation is primarily a reaction to the invasion of *Bacterium tumefaciens*. The previous experiments were conducted on annuals, biennials, or deciduous trees, in which the period of growth of the host as well as of the crown gall is normally interrupted. In these experiments, some of the galls are benign to the host and behave in a manner more analogous to a scar, a cheloid, than to cancer. In a comparatively small percentage of cases the galls act as true malignant tumors. The parts of the inoculated stem become necrotic above and even below the point of inoculation.

The present investigation was conducted on the rubber tree (*Ficus elastica*), which is an evergreen perennial plant and grows indoors, so that the crown galls may be watched for long periods of time without interference by secondary contaminations. These experiments have shown that twelve months and more after inoculation, nearly every crown gall produces a necrosis of the inoculated branch, though at first the gall may attain a large size without apparent injury to the host-branch. Then frequently, without any additional increase in the size of the gall, the branch becomes necrotic both below and above the gall. This necrosis increases in a centrifugal direction from the gall; thus the tip of the branch may still be alive while a part of the branch below the gall is necrotic.

These phenomena cannot be due to impairment of nutrition since the crown gall frequently attains its largest size without a concomitant

necrosis, and the latter is always subsequent; nor can the necrosis be due to the action of some toxic substances produced by the crown-gall cells. It is difficult to conceive of such a rapid change in the metabolic chemical functions of a cell. The most plausible explanation is a change in the reactivity of the host tissue.

For a time the *Bacterium tumefaciens* produces a progressive reaction in the injured and surrounding tissues of the host, which manifests itself in proliferation of cells and formation of a crown. Sooner or later this is followed by regressive reaction which manifests itself in progressive necrosis. Since plants lack the lymphoid tissues and cannot react to parasitic invasion by inflammation, the above described methods of reaction are the only possible ones.

Thus, the neoplasia in the crown-gall disease is primarily a protective reaction of the host tissue to the invasion by *Bacterium tumefaciens*. The morphological studies by the writers of two other parasitic diseases, clubroot of cabbage (*Plasmodiophora brassicae*), and potato-wart disease, or potato cancer (caused by *Chrysophlyctis endobiotica*) show that identical reactive cell proliferations and formation of new growths may be caused by other parasites besides *Bacterium tumefaciens*.

It is possible that in most parasitic diseases of plants both neoplasia and necrosis take place, though the former may be so insignificant and transitory that it evades detection.

DISCUSSION

Dr. Wells: It is pleasant to hear that potatoes and cabbages contain items of interest besides calories and vitamins. This subject is, of course, of great importance, because the extremely interesting work of Dr. Smith has attracted so much attention, and there has been a lively discussion as to whether these processes in plants are really to be considered as true neoplasms. I wish to express my personal appreciation of this contribution because it must be determined whether these things in plants are true tumors or not, since their interpretation will have much bearing on our evaluation of the work in cancer research.

5. A PHASE OF TUMOR BIOLOGY

Dr. Frederick Prime (New York):

SUMMARY

In the past few years much confusion has arisen on account of the various results reached by different cancer investigators doing practically the same line of research. A great deal of this confusion is probably due to the use of tumors whose biological characteristics are really unknown to the investigator. Applications are made to this laboratory every year by workers on the cancer problem who desire a carcinoma or sarcoma which they wish to propagate for a short time in

order to carry out some investigation. They then report their results on a few dozen animals, whereas if the characteristic action of the tumor over numerous generations had been known to them, or if they had used a larger number of animals their results would have been very different. For instance, the Jensen rat sarcoma is reported in one paper to take in almost 100 per cent of animals inoculated. In running back over our books for the last six years we find that in 5400 animals surviving at the end of three weeks we have only one series in which there was 100 per cent of takes, and only a few in which the takes were as high as 90 per cent. If our experiment had been done in March, 1914, for instance, we should have had 100 per cent takes in our controls, whereas if it had been done in July, 1916, we should have had only 20 per cent positives. In another instance important conclusions were drawn from two series of tumor 63, one with a high percentage of takes, the other with no takes, but our books show that the same fluctuation has occurred in routine transplantation. The results vary from month to month and from year to year, but if the averages for the months are made the variations are less marked. Of all our tumors, the mouse sarcoma Crocker Fund No. 180 gives the highest percentage of takes and is the most consistent, the number of takes never having fallen below 85 per cent. The Ehrlich mouse sarcoma is a close second to this and in only one month did this fall below 80 per cent of takes.

Certain members of our staff, I myself among them, have had the general impression that our tumors grew less well during the summer months, but upon analysis this proved to be quite incorrect; the death rate among the animals may have been higher, but the tumor growth rate did not show any such change. For six years the Jensen rat sarcoma had its highest number of takes in August, and its lowest in July. The Flexner rat carcinoma had the highest number of takes in April, and the lowest in November. It behooves us, therefore, to know and study our tumors carefully before starting any investigations on them, and to recognize the fallacy of using small numbers of animals in arriving at a conclusion.

DISCUSSION

Dr. S. R. Benedict (New York): I think it would be interesting if Dr. Prime could give some more definite statements as to how much these fluctuations would influence conclusions, that is, whether the results would be diametrically opposed through an increased number of animals, and whether he would recommend that an experiment be based on 5000, or on 500, or on 50 animals. A general criticism of this type calling attention to the number of animals might be misinterpreted unless it is made more specific. In the matter of transplanting a given tumor, for instance, we have quite exact data, and if we transplant from an experimental animal, using the same tumor, we should get a similar growth in both series, except for the experimental factor.

I should like to ask how large a series would have to be, and whether it must be repeated for several months before definite conclusions can be drawn.

Dr. Prime: I think the criticism is exceedingly pertinent. We feel that a good deal of confusion arises with men working along the same line who report such varying results from exactly the same tumor, because they do not quite know the biology of the tumors. One may report results with a certain series or a certain tumor and another give data of an opposite nature, but that does not necessarily mean that one or the other is incorrect. If a little more were known about that tumor, it would probably be found that neither was making very great errors. I think one advantage of a laboratory of this kind is that we have tumors in such large series, and that our records are always available, so that anyone can refer to a tumor with which he is working. But so many of the people who come here for tumors do not care what they get or what happens to it. They want a tumor they can grow in one or two generations. I do not think conclusions should be drawn from experiments with 25 or 50 animals. I think each series should contain one or two hundred animals, and that one series alone should not be taken as a basis for conclusions. As you see here our results vary from month to month; and if experiments were made on several series and the results averaged, I think they would be very much more valuable than are deductions drawn from one or two small groups, as is often done. It is simply that we want to avoid the error of making too sweeping conclusions from too few animals and from a lack of knowledge of the growth rates of these tumors. I think that eventually if experiments are made in this country with tumors whose growth and behavior is known, and with animals whose behavior is known, results from the various laboratories would be very much more consistent.

Dr. Wood: The question has other phases, also, such as the statistical study of tumor regression after taking and the study of the tumor growth rate. In some instances the number of takes is most important, for instance, when attempting to establish immunity against a tumor it is vital that we know that the tumor is not one which establishes immunity against itself. Some workers have reported experiments in which they claimed to have established immunity by means of treatment of the animals, but they overlooked the fact that the tumor used was one which immunizes against itself. In experiments in which attempts are made to influence the growth of a tumor by therapeutic means, it is necessary to use a tumor in which the number of spontaneous disappearances is negligible. Our no. 180 is such a tumor, and because of this fact and because of its easy inoculability, we usually supply it to those who wish to make therapeutic tests. One naive experimenter, after working with this tumor, wrote that he wished we would send him a better one, because although the tumor

he had previously used was curable by his treatment, this no. 180 was worthless because it was too resistant. The value of his cancer cure is evident.

As to the number of animals to be employed: we are in the habit of using small series of animals over a long period of time; instead, for instance, of using three hundred animals for an experiment, and one to three hundred as controls we use two sets of twenty-five animals each, and then repeat the experiment several times, employing a variety of types of tumor, both carcinoma and sarcoma. We thus get long series which can be compared with each other. A few months ago there was published a statement concerning certain effects of *x-ray*, the conclusions being based on the fact that in twenty or twenty-five animals, treated in one way, there were no tumor takes, while in the control animals there were 80 to 90 per cent of takes. It was, therefore, assumed that an immunity was proved. All it was necessary to do to show that the point was not proved was to open one of our record books of the tumor this experimenter was using and find a page on which were listed twenty-four animals which had been inoculated with the same tumor on the same day, without a single take, while the next lot of twenty-four under the same conditions showed 60 to 70 per cent of takes. The only way to avoid these errors is to repeat the experiment time after time, not necessarily with a large number of animals, and in this way eliminate random fluctuations of which we know nothing, but which are due to the fact, too often forgotten, that an animal is not a test-tube, and that the tumor graft, also, varies in its biological activities.

As to the question whether or not we can stimulate or retard the growth rate of a tumor, the evidence which we have accumulated proves that it is impossible to draw any accurate conclusions as to rate of growth. Some 3000 grafts of tumor no. 180 were planted in healthy mice from one dealer, allowed to grow for three weeks, and then excised and weighed. It was assumed that the average weight of these tumors would furnish a useful standard, yet in the next series of 200 tumors the average weight was found to be double that of the previous 3000. When precautions are taken to have the site of inoculation, the strain, the weight and the age of the animals, and the method of feeding the same in all cases, and yet great fluctuations occur, we are justified in saying that no conclusions as to growth rate can be drawn. As the result of experiments in which only a small number of animals was used and no controls were kept, some observers have claimed to have stimulated tumor growth by chemicals; but while such claims may be correct, they are absolutely unproved because there are no available measurements by which growth rate can be determined.

These are some of the complications of this tumor work, and the only way to eliminate them is to know the biological qualities of the tumor we are using, and to adjust the numbers of animals used to the conditions of the experiment.

Dr. Stone: It seems to me that the purpose of this presentation is an exceedingly good one. It brings to my mind the experience we are repeatedly having at the Memorial Hospital in regard to radium. Men go to Pittsburgh, for example, and make a contract for radium. Then they come to the Memorial Hospital to learn how to use it; and after a few days they return to Pittsburgh and get their radium. The conclusions drawn from their work certainly can be no more accurate than those which are drawn from the observation of the effects of therapeutic measures on a few animal tumors.

6. THE INFLUENCE OF CERTAIN DIETS UPON TUMOR SUSCEPTIBILITY AND GROWTH IN ALBINO RATS

Dr. K. Sugiura and Dr. Stanley R. Benedict (New York):

SUMMARY

Curves constructed so as to show the linear and the percentage growth of the fetus and of the Flexner-Jobling rat carcinoma in normally fed animals indicate that there is quite close agreement in growth between the two (the carcinoma and the fetus).

The experiments reported were designed to determine whether general differences in diet influence tumor growth independent of special deficiencies, and to study the effect of certain specific deficiencies in diet upon tumor susceptibility and growth. A complete diet, composed of banana, 83 per cent, yeast, 0.5 per cent, and protein-free milk, 0.5 per cent, was found to yield the same percentage of successful inoculations and the same rate of growth as was secured with a diet of wheat bread and whole milk. The elimination of certain accessory factors in the banana diet was found not to influence tumor growth except when the diet was restricted wholly to bananas. In this latter case, the percentage of successful inoculations was the same as in control animals, but the rate of growth of the tumor was markedly retarded. Such dwarfed tumors showed no histological changes from the normal, and resumed normal growth when the animal was placed upon a normal diet.

DISCUSSION

Dr. Wells: Dr. Benedict's paper giving the curves of fetus and tumor growths recalls a discussion which I heard when a paper from Ehrlich's laboratory was presented. Some German, with a mathematical turn of mind had figured the rate at which tumor cells grow. At the end of a certain number of days, he said, one would have enough tumor to inoculate ten more animals, and at the end of three years the total tumor tissue would have formed a cube so large that it would take a ray of light 105 years to pass one side of it. Some of the biologists present said there was nothing remarkable in that, because fetal tissues had been demonstrated to have a fully equal capacity for growth.

Dr. Wood: I did not know when I spoke of tumor growth rates that Dr. Benedict was going to present anything which turned upon the details of growth rate, and I still hold the same views that I expressed a few minutes ago. My opinion of Dr. Benedict's experiments is that they may be right and they may be wrong. The only way to prove them right is to take the two significant series and repeat them on a group of twenty-five rats, some ten times. This will eliminate random fluctuations. Then if the averages show a striking difference between the dieted and the control groups the fact may be accepted that diet influences the type of tumor used, though not any other tumor, and especially not a primary tumor. I can take from my own record books pages that show very much diminished tumor growth rates running over a period of two or three months, followed by a period of very abundant growth, although the animals were untreated. I cannot say, therefore, that Dr. Benedict is right or is wrong and the only way to get proof either way is to repeat the experiment under a great variety of conditions. It is the same problem as the question whether radium does or does not stimulate a tumor. Many workers believe that threshold doses of radium do stimulate but I have been unable to prove it.

7. THE EFFECT OF COMBINED HEAT AND RADIATION UPON TRANSPLANTED ANIMAL TUMORS

Dr. George L. Rohdenburg and Dr. Frederick Prime (New York):

SUMMARY

The investigations of Loeb, Stevenson, and others have shown that neoplastic cells are killed in relatively short periods by comparatively low degrees of heat. In the present experiments mice and rat tumors were cut into small fragments suitable for inoculation, exposed to varying degrees of heat for varying periods while in Ringer's solution in a water-bath, and then inoculated into animals, the lethal effect of the manipulation being indicated by the percentages of takes in the inoculated animals. In this fashion exposures to 40, 41, 42, 43, 44, 45, and 46°C. were made for 15, 45, 75, 135, and 195 minutes. The lethal effect of heat was manifested first with 40°C. at the end of 195 minutes, and with 41°C. at the end of 75 minutes; with 44° there were only 35 per cent of takes at the end of 45 minutes.

Bovie has shown that albumins which have been radiated coagulate at much lower temperatures than those not radiated, and it was thought it might be possible to apply this principle to the killing of the cancer cell. Fragments of tumor prepared as were the fragments used in the heat experiments were exposed to x-ray for periods varying from 10 to 30 minutes, 30 minutes representing three erythema doses. Directly after radiation they were exposed to varying degrees of heat after the method outlined in the previous paragraph and then inocu-

lated into animals. It was found that whereas heat alone at a given temperature was not lethal, and x-ray in a given dosage was also not lethal, the two when combined were lethal.

In another series of experiments the process was reversed, heat being applied first, and radiation afterwards. The same effect was noted, the sequence being apparently immaterial.

These results suggest a new method for treatment in human cases, and experiments are now under way in which a practical application of the principle is made on both human and spontaneous tumor animal material.

DISCUSSION

Dr. Greenough: We have felt very strongly that the observation of Dr. Bovie in regard to heat sensitization was one of possibly very great clinical importance, but that further work should be done along experimental lines before any serious attempt is made to apply it to living patients. The point of the greatest importance is that a degree of heat which under ordinary circumstances can be withstood perfectly well by the individual cell results in the death of that cell if it is applied after radiation. The temperatures which Dr. Rohdenburg has indicated on the charts apparently are such that a definite effect can be obtained in a certain small percentage of cases from the temperature alone; to that extent, therefore, the experiment is not quite comparable to Dr. Bovie's original observation; but I am very much interested in the results, and I feel that without question this opens up a field for clinical application in the actual treatment of cases.

Dr. Wood: I think everyone realizes now that we have reached a point where we can say definitely whether or not radium and x-ray will cure any malignant tumor. The only question is will the patient survive the dose. In other words, we have come to a point where it is a question of operative mortality as compared to x-ray or radium mortality. It is perfectly possible to obtain the absolute destruction of cancer cells by a sufficient quantity of x-ray; and the same thing can be done with radium. The only question is the practical application. In my opinion, at the present time most internal tumors require so much radiation that serious, very often fatal, damage to the normal organs would inevitably be inflicted. For example, the lethal dose to a carcinoma cell of a tumor 10 cm. below the skin would be between sixteen to twenty erythema doses, if a highly filtered x-ray were used. I have seen quite serious general disturbances to the intestinal tract result from two erythema doses. The recent German literature, also, contains reports of a number of deaths, some apparently from intestinal obstruction, following heavy doses of x-ray. The problem is to kill every cancer cell, although it is recognized that much benefit can be obtained by the destruction of the main portion of the tumor, the central areas, for example, which are poorly vascularized and easily

subject to necrosis from thrombosis of the capillaries of the tumor. But the destruction of the peripheral portions of the tumor, which are well vascularized, is quite another matter. Hence, a very large dosage is required to kill absolutely all the tumor cells, either in the test-tube or in the animal itself. Thus, although a point has now been reached where we can say definitely that cancer can be cured by radiation, with the dosage determined experimentally in this laboratory, the question still is how we can also save the patient's life. With this point kept clearly in mind Dr. Rohdenburg started his experiments. The original experiments Dr. Prime and I began some four or five years ago. The lethal death-points for cells were determined and are standard, so there can be no difficulty in reproducing these results anywhere if tumor 180 is used. An x-ray machine can be calibrated biologically. My clinical experience shows that in a rapidly growing carcinoma in man the death point is about the same as in 180. The slow growing squamous-cell epitheliomata seem to have extra resistance, owing to the ability of the cells to cornify and practically to enter a resting stage. It is now accepted that the lymphosarcoma and many of the bone tumors are more susceptible to x-ray than are the very rapidly growing highly malignant carcinoma or sarcoma. The maximum practical dose of x-ray or radium which the patient can survive remains to be determined, but if it is remembered that there is, after all, a surgical mortality and that untreated cancer is fatal, we are justified in giving radiation pretty close to the limits. Most radiation treatments are far beneath the killing dose for the cells of most of the internal tumors. I do not believe that the induced connective tissue reaction around the tumor cells kills them. It may encapsulate them for a while, but usually the cell enclosed with scar tissue finally begins to grow and destroys the patient. After having given the maximum dose of x-ray which the skin of the patient can stand, we might be able by thermo-electrical devices to apply heat which does not seriously damage the skin and which produces death of the cells. The morphology of cells killed by heat is practically the same as that of cells killed by x-ray or radium, and I think that this paper is important from a therapeutic point of view, as well as a scientific one. It may be possible by some high frequency method to add to the semi-lethal dose of x-rays a semi-lethal dose of heat without causing the death of the patient and that, I think, these experiments clearly show. The practical application will require long careful study on human beings.

Dr. William Duane (Boston): In regard to the practical application of heat and x-rays, this was tried very extensively in Paris seven years ago, and there was claimed a good deal for the combination. There was one difference between that procedure and the one reported here, the difference being that the two destructive agents were applied at the same time. Keating-Hart warmed the human tumor tissues and at the same time applied x-rays, and I understand that here heat was

applied either before or after radiation. It would seem probable that the application of the two at one and the same time would produce a greater effect. As regards the ability to destroy any tumor by radiation, if an intense enough radiation is used almost any organic compound can be destroyed. Water can be decomposed by the action of the rays.

Dr. Rohdenburg: I would like to say that these experiments differ from those previously reported in that lower degrees of heat were used over a much longer period. In the recent German literature the combination is reported to have been tried, but tried by combining diathermy produced by seven amperes of current for a period of thirty seconds. This dosage produces coagulation necrosis and a typical burn. Our aim is to obviate this factor of burn. The tumors we have treated with high frequency, using 25 milliamperes per square inch of electrode surface and continuing the application for twenty minutes, show scarcely any recognizable change in the tissue for a period of a week or ten days. This dosage produces a temperature between the electrodes of 40°C., or 104.8°F. which is very readily withstood by any normal tissue, and many degrees below the temperatures produced by Keating-Hart or the more recent investigators.

8. INTERSTITIAL INJECTIONS OF AN ACTIVE DEPOSIT OF RADIUM EMANATION IN A RAT CARCINOMA

Dr. Halsey J. Bagg (New York):

SUMMARY

In this experiment a definite attempt was made to use an "active deposit" of radium emanation as a local agent, employed in the form of a solution, to control the growth of an experimental rat tumor—the Flexner-Jobling rat carcinoma. The writer gave a preliminary report of the results of treating twenty-one animal tumors.

Interstitial injections of a radio-active salt solution of radium emanation were found to retard materially the growth of the tumors and in some cases to cause their regression, resulting in characteristic cellular changes in the tumor tissue. Definite characteristic, histological radium changes were noted—frequent hyperchromatic and homogeneous nuclei, cellular hydrops, fairly extensive central necrosis, and a terminal onset of fibrosis.

It was found that comparatively large doses of radium could be injected into rat tumors without the escape of the radio-active solution into the surrounding tissues. (A comparatively small intravenous or subcutaneous injection of the same solution invariably results in severe reactions.) The rapid decay of the radium when used in this form, in addition to the walling-off effect of the tumor capsule, appears to be sufficient to confine the radium action to a desired zone of tissue. This was an encouraging observation from the standpoint of human therapy.

DISCUSSION

Dr. Duane: I think it would be well to emphasize the very great danger of this method of using radium or radio-active substance. The alpha rays are utilized, and since of the total activity of all the beta, gamma, and alpha rays, about 90 per cent is due to the alpha radiation, it is evident that the alpha rays produce relatively tremendous effects. It is well to bear in mind, therefore, that this method of treating tumors should be employed with very great care. Over 100 millicuries of deposited activity would be a dangerous dose for any human being.

Dr. Benedict: I should like to ask if this is not simply a question of the size of the tumor mass; that is, one might destroy a small tumor, but affect only the center of a large one, making it necrotic.

Dr. Stone: It seems to me that both of these methods, the intravenous injection of the active deposit and the local infiltration of the tumor, represent a type of experimental work which is most desirable, but, as Dr. Duane has just said, we must use extreme caution in applying this practically. The general constitutional effects from intravenous injections are very marked. So far we do not know the constitutional effects from the infiltration method, because it has not been applied except in two or three instances, and then very cautiously. It does seem, however, as if we might make use of the alpha rays in this infiltration method, which, as Dr. Duane has said, comprises so much of the energy of radium, and which apparently we cannot use in any other way. There is another point in regard to the practical application of this infiltration method. Personally I dislike to think of radium used either by this method or by the surface application to the extent that we get actual destruction or caustic effects. I conceive of using radiation for a strictly biological effect, without causing absolutely local destruction, for years prior to the use of radio-activity all of us had experience with the use of the cautery, and we knew just how much tissue we could destroy with that. With the use of radio-activity we do not know just how extensive the destruction is, how long it is going to continue, or what the destructive effects upon the normal tissues in the neighborhood of the tumor are. The possibilities of destruction are so great that we must use extreme caution. In making a practical application of this infiltration method I think we must begin with the very smallest dose so as to get the so-called biological effect, and not a local caustic effect on the tumor itself.

Dr. William B. Coley (New York): The work of Dr. Bagg is extremely interesting and valuable, but I believe that the cautions given by Dr. Stone and Dr. Duane are timely. At the Memorial Hospital Dr. Janeway has used the method in a considerable number of cases in human beings, beginning with small doses and increasing to fairly good sized ones; but so far none of the results gives us reason to believe that

it is likely to be of permanent value as a therapeutic measure. I believe, as Dr. Bagg points out, that the cases in which good results are obtained are those in which the tumor is localized by the surrounding tissues, and if that is so, why can not one use the bare tubes which will remain localized, and not get into the tissues and damage the kidney, liver, etc.? I believe with Dr. Wood that we can always give enough radium to kill the cancer cells, but not in most deep seated cancers without killing or injuring the patient.

Dr. Bagg: Dr. Benedict has brought up the subject of the relation of tumor size to the subsequent reaction to the treatment. I have found that in most cases the central portions of the treated tumors were necrotic, and Dr. Ewing, who has seen my sections, has called my attention to the peculiar manner in which the radio-active solutions have diffused to different parts of the tumor, causing more extensive reactions in certain areas of the tumor than in others. I have tried in some recent work to infiltrate the tumors from within, by first placing the hypodermic needle in the center of the mass and then moving it to different parts of the periphery of the tumor, and injecting a small amount of the solution in each place. In regard to the apparent increase in size of some of the treated tumors, I would say that this is no doubt due to the production of an edematous condition and the accumulation of fluids in the center of the tumor, in which case the ring of tumor tissue was crowded to the periphery, while the actual bulk of the tumor tissue was no greater than the amount present at the beginning of the experiment.

9. FACTORS UNDERLYING SUSCEPTIBILITY TO A TRANSPLANTABLE TUMOR IN MICE

Dr. C. C. Little:

SUMMARY

A sarcoma, J. W. B., which originated in an inbred race of Japanese waltzing mice grows upon transplantation in 100 per cent of the animals of that race inoculated. It fails to grow progressively in more than 99.5 per cent of the common non-waltzing stock mice inoculated.

Beginning two weeks after inoculation each animal is observed at weekly intervals, and the presence or absence of growth is noted. When a growth is present the animal is recorded as +; when no growth is found, it is recorded as -. Two groups of animals have been observed. They are (1) ordinary non-waltzing stock mice series (N.); (2) back-cross hybrids (B. C.) produced by crossing an F1 generation hybrid between Japanese waltzing and common non-waltzing mice back with the common non-waltzing parent race.

The present series of experiments deals with growth of the tumor from the second to the sixth week after inoculation, inclusive. Many

of the animals showing a growth at the sixth week observation showed eventually regression and disappearance of the tumor. The factors studied are, therefore, those allowing the initial six weeks' growth of the tumor. This growth may or may not be continued later, depending upon the hereditary constitution of the mice used. The animals inoculated were divided by age into ten groups, respectively, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 or more days old at inoculation. For each of these groups the percentage showing growth at 2, 3, 4, 5, and 6 weeks after inoculation was determined. The results of *all* groups according to weeks after inoculation were as follows:

WEEKS	N., PER CENT +	B. C., PER CENT +
2	16.1	27.1
3	13.9	21.0
4	10.7	12.4
5	7.8	13.6
6	5.3	13.1

It will be noted that the percentage of animals showing growth in the N. series steadily decreases, while in the B. C. series, there is an initial decrease followed by a rise to a level at the fourth week at or near 13 per cent. The total per cent for the N. series is 11.12 ± 46 and for the B. C. series is 17.54 ± 83 . The difference is 6.42 ± 95 and is, therefore, significant, being 6.7 times its probable error.

Studied by age groups with the sexes combined, there is seen to be a distinct difference between the two groups.

AGE AT INOCULATION	N., PER CENT +	B. C., PER CENT +
<i>days</i>		
2 to 10	12.87 ± 0.6	13.77 ± 1.05
12 to 20+	9.45 ± 0.6	21.58 ± 1.28

While in the N. series the *lower* age group shows the most growth, exactly the opposite is true of the B. C. series in which the higher age group shows a significantly higher percentage of growths.

When the sexes are studied separately the males of both series and of both age groups are apparently not significantly different from each other. The females, on the other hand, show a significantly *lower* percentage of growths in the *higher* age group of the N. series and a significantly *higher percentage* of growths in the *higher* age group of the B. C. series. The higher age group of both sexes is more mature and further differentiated than is the lower age group. Within the higher age groups many of the females become sexually mature during the period of observation. This gives to them an additional chance for differentiation and the assumption of sex limited or other biological

characters dependent upon their hereditary make-up and upon the degree of differentiation which their tissues have reached.

AGE AT INOCULATION	N.		B. C.	
	♂	♀	♂	♀
<i>days</i>				
2 to 10	15.70±1.64	19.46±1.31	14.51±1.51	12.12±1.76
12 to 20+	10.30±1.20	9.39±0.87	15.38±1.55	25.74±2.07

What we are observing, therefore, is a racial difference. In the N. series a steady decrease in percentage of growths, especially marked in the females, is found. In the B. C. series there is at first a decrease followed by a rise, a level of about 13 per cent growth being reached. This level is due for the most part to the presence in the B. C. generation of animals which will show permanent progressive growth of the tumor. The results of both series coincide with and amplify the explanation of the hereditary nature of susceptibility to the J. W. B. tumor, advanced by Tyzzer and the writer in 1916.

A STUDY OF A LIPOMYXOSARCOMA WITH COMMENTS UPON THE ORIGIN OF THE FAT CELL

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Neoplasms of mesenchymal origin are frequently the cause of much controversy owing to a lack of differentiation into tissue which would permit their being placed in a definite organoid category. The term "liposarcoma" has been used to denote malignant change in the connective tissue of a lipoma, itself one of the most benign of tumors, and also to accent the fact that the growing fat cells have invaded the surrounding tissues or metastasized. A tumor which presents the accepted criteria of malignancy, derived from fat cells which are atypical in size, shape, and staining characteristics, with many mitotic figures and invasion of septa and capsule, is of the greatest rarity, and a study of such a growth may throw light upon the origin of the fat cell, regarding which there is much conflicting opinion. The writer has lately had the opportunity to examine a tumor of that type, removed from a patient in the Surgical Out-Door Department of the Peter Bent Brigham Hospital, and with the kind permission of Dr. Harvey Cushing a brief summary of the case is given.

The patient, a Russian Jew, came to the hospital on November 18, 1920, complaining of a growth in his right thigh. His past history is unimportant except in so far as it concerns the tumor. In 1917 he noticed a firm swelling the size of a walnut beneath the skin on the mesial surface of the right thigh. It caused no pain. A few months later it was noticeably larger, and massage was instituted for a short time. It continued to grow slowly and became a little painful, particularly in cold weather. He was, however, able to work in his store, and he walked into the hospital.

Physical examination was negative except for the local findings. On the inner surface of the upper third of the right thigh, beneath the skin and extending deep into the muscles, was felt a somewhat movable, irregularly rounded growth, about 18 x 6 cm. in size, and not tender. There were no palpable nodes. On November 19, under novocaine anesthesia, the growth was removed by Dr. A. H. Brewster. It seemed to have three distinct capsules, but there was no evidence of invasion of the surrounding muscles. The outer two capsules were thick and fibrous, and stripped away readily from the more delicate layer covering the tumor. Most of the blood-vessels entered through a sort of pedicle on the deeper portion of the tumor. The dead space was closed with catgut sutures and the skin with silk. On December 21 the wound had entirely healed. On February 3, 1921, the wound was in good condition, with no evidence of local recurrence or metastases, and the patient felt perfectly well.

Pathological examination. The specimen consists of an irregular mass measuring 17 x 8 x 5 cm., and weighing 510 grams. It is surrounded by a delicate transparent capsule beneath which ramify a moderate number of dilated blood-vessels. The larger vessels converge upon a narrow zone on one side which probably represents the pedicle where the vessels were clamped off. It is uniformly firm, more so in slightly depressed areas between the bulging large lobules into which it seems to be divided. A longitudinal incision shows it to consist of two main types of tissue. About three-fourths of the tumor is pale, rather pink, and opaque, with the consistence of a very cellular tumor. The mass is divided into more or less definite lobules by fibrous septa of varying thickness. A broad fibrous band apparently invaded by streaks of tumor separates it into two main parts, which are in turn subdivided into smaller lobules by narrow vascular septa. More or less well circumscribed areas suggesting mucoid degeneration are present in the middle portion, irregular wells of mucilaginous fluid bathing the surface like honey in a comb. The fluid gives the characteristic reactions of true mucin.

Microscopical examination. Tissue was fixed in 10 per cent neutral formalin and in Zenker's fluid. Frozen sections were stained with Scharlach R and hematoxylin, other preparations with methylene blue and eosin, phosphotungstic acid-hematoxylin, aniline blue-acid fuchsin, osmic acid, Mayer's mucicarmine and mucihematein, and Verhoeff's elastic tissue stain. A study of the sections gives the following facts.

The tumor is surrounded by a thin compact layer of connective tissue in which are a moderate number of elastic fibers. The capsule

sends prolongations into the tumor dividing it into smaller units. Elastic fibers and thin-walled arteries and veins accompany the septa and many fine capillaries are present in the stroma, which consists of a delicate collagenous reticulum in which the tumor cells lie, and which appears to arise for the most part from the blood-vessels. The tumor is very cellular except in small areas which have undergone mucoid change, where the tissue suggests that of the umbilical cord. In shape the cells vary from medium sized spindle cells through intermediate forms to the more or less round outline of mature fat cells. The nucleus follows the general shape of the cell and has a densely staining nucleolus. Practically all the cells contain refractive fat droplets which stain with Scharlach R and reduce osmic acid. In the spindle cells the droplets are, as a rule small, almost always smaller than the nucleus, and tend to localize in the vicinity of the nucleus. These spindle cells often have delicate wavy processes of some length. In the intermediate, polyhedral forms the fat drops become larger and coalesce, the nucleus stains more deeply, and mitotic figures are seen in large numbers. The dividing cells have a large fat content. The more mature round cells are distended with a single droplet of fat which occupies practically the entire cytoplasmic space and flattens the nucleus, giving the typical signet-ring appearance. Many of these cells have ruptured, and the fat has escaped into the interstices, where it has formed little pools. Invasion of the capsule and septa by the tumor cells has occurred. No intravascular growth is found, however. Scattered through the stroma are also many mast cells, none of which contain oxidase.

Sections from the areas of "mucoid degeneration" show an abundance of loose wide reticulum containing mucin. In this are present a moderate number of tumor cells of all types with large and small fat droplets. With specific stains mucin can be demonstrated in most of the cells in the form of a fine or coarse granular deposit in the cytoplasm, clustered about the fat droplets. Mucin is present in small amount in the cells of various other parts of the tumor but is most abundant where it exists in macroscopic quantities.

DISCUSSION

A lipoma is a very common and benign tumor composed of mature fat cells. A liposarcoma is much less frequently met with and in a large percentage of cases has shown areas of myxomatous degeneration. Knox (1) in 1919 collected twenty-

five cases and added two more. The names lipomyxosarcoma, myxoliposarcoma, liposarcoma myxomatodes, and myxosarcoma lipomatodes have been used by various writers, depending upon which tissue was in excess, the fatty or mucoid, or whether the writer regarded the mucous tissue as a product of degeneration of the fatty tissue or vice versa. "Sarcoma" denoted invasion, recurrence, or metastasis. Fibrous tissue, cartilage, and bone have also been found in these neoplasms, making them rather mixed tumors, although with all elements derived from the same germ layer. Hirsch and Wells (2) studied a retroperitoneal tumor of 69 pounds, parts of which were pure lipoma, others fibrosarcoma. In none of the cases described have mitotic figures been found in the fat cells, although evidence of direct division has been noted. The most frequent sites of these tumors are the retroperitoneal tissues, the muscular fasciæ of the thigh and leg, the mesentery, the cheek, and the breast.

The tumor forming the basis of this paper has several unique features. The bulk of it is composed of cells in practically all of which is much fat. Most of these cells are of the embryonal type, being spindle or stellate with delicate fibrillary processes. Many transitional forms are present, up to and including the large round signet-ring mature fat cell. Many cells are in mitosis and these also contain fat droplets. About one-fourth of the tumor is bathed in mucin, and the cells in such places contain both fat and mucin.

In offering an explanation for the presence of mucin and fat in the same cell, we must go back and consider the embryological development of the connective tissues. The connective tissues which include fat and mucous tissue are derived from the mesenchyme. Todd and Bowman (3), in 1845, were among the first to suggest that adipose and areolar tissues are distinct and independent. Kölliker, in 1856, described the original fat cells as glands, such being found in the axillæ and mesentery, and around the kidneys. In 1870 Toldt's (4) investigations led him to the conclusion that the "glands" of Kölliker are the source of fat, and he called them "fat organs." Flemming



FIG. 1. PHOTOGRAPH OF THE ORIGINAL TUMOR, EXTERIOR



FIG. 2. PHOTOGRAPH OF THE TUMOR, OPENED BY LONGITUDINAL INCISION

The bulk of the tumor is pale pink, opaque and firm. The crosses (X) mark areas of myxomatous tissue.

(5) in 1876, in a comprehensive study, advanced the opinion that the ancestral tissue of fat is a fibrillar connective tissue. Hammar (6) also held this view.

In 1894, Borden (7), in a comparative study of the fat cell, stated that (i) in the lower vertebrates the fat cell is developed from one form of cell arising from special centers; (ii) in the higher vertebrates the fat cell is developed from two forms of cells differing greatly in size and shape; and (iii) one of the original fat cell forms in the higher vertebrates is homologous with the special center forms of the lower vertebrates. This cell is gland-like and in no way resembles the connective-tissue cell; the other form, while closely resembling the connective-tissue cell, is most probably a special cell derived from the special center forms by cell division and "migration metastasis." Multiplication is by indirect division and the cells never turn into connective tissue cells.

Bell (8), working with material from a calf fetus and from steers, described a peculiar open-meshed "pre-adipose" tissue which forms well defined lobules before true fat cells appear. This "pre-adipose" tissue consists of loosely arranged cells with two or more coarse processes. The branched cells may contain fat droplets a long time before they assume the rounded form. Bell supports Flemming in the belief that the pre-adipose tissue is clearly a fibrillar connective tissue. He also described how the cells nearest the blood-vessels filled with fat first, the processes becoming absorbed. Altmann's granules were observed when the cells were yet branched and before the first fat droplets had appeared.

In Lewis and Stöhr's Textbook of Histology (9) it is stated that in the four-month embryo the fat cells are quite like the surrounding fibroblasts, being fusiform or stellate and containing vacuoles or droplets. Mallory (10) emphatically says that the fat cell is a perfectly definite type of cell formed by differentiation from a mesenchymal cell, that it is not a fibroblast, does not arise from one, and, when it undergoes atrophy, does not turn into one.

The earlier writers, in speaking of the connective tissue cell, probably referred to what is now termed the fibroblast. Consequently, at the present time the opinion is strongly held by many observers that the fat cell is essentially a modified fibroblast, whereas others assert that it cannot be so considered. I believe that the demonstration in this tumor of mucin in the cells which otherwise appear as fat cells supplies an argument in favor of the former idea.

Mucin is a slimy substance produced by certain epithelial (beaker) cells and by the fibroblast under special conditions. We are concerned here with its production by the latter cell. The umbilical cord furnishes the classical example of mucous tissue. The mucin is said to be secreted by the fibroblasts without the formation of special granules or vacuoles (Lewis and Stöhr). Cohnheim restricted the term mucin to those substances which are excreted by epithelial cells, and reserved the name mucoid for closely allied bodies which occur in various organs of the body, such as the vitreous humor, cornea, umbilical cord, and tendons.

Mucin has certain characteristic chemical and physical reactions which permit its identification, so that the term mucoid seems unnecessary except for describing gross physical qualities of the fluid. It is not produced normally in the adult, but in tumors of mesenchymal origin it is found not infrequently, as in myxoma, fibroma, chondroma and osteoma, and in the more rapidly growing myxosarcoma. Ewing (11) states that mucous tissue "is genetically related to fat tissue into which it is extensively transformed during normal growth. . . . It does not appear that true myxomas ever tend to differentiate into fibroma or lipoma." He also asserts that the spindle and star cells in the mucous matrix may contain hydropic or fatty droplets.

The question naturally arises, is the substance in the tumor cells which stains with Mayer's mucin stains really mucin? The tissue in which these cells are found in greatest numbers is bathed in a slimy fluid which gives the accepted tests for mucin and likewise stains with mucicarmine and mucihematein.

It is, of course, impossible to be certain that the intracellular substance is mucin, yet it stains "specifically" and any other interpretation would seem illogical. The cells otherwise have the appearance of young fat cells such as are found in other parts of the tumor.

It might seem unwise to attempt to settle the origin of a cell solely by study of a tumor derived from that type of cell, particularly in view of the frequency of metaplasia in pathological experience. But metaplasia apparently has a very narrow scope, its best illustrations being in epithelial reactions, and application of the principle to the connective tissues has led to great differences in interpretation. To explain the secretion of mucin by the cells of this tumor as an example of metaplasia does not seem as rational as to assume that the cells which are essentially fat cells take on the characteristics of a less differentiated tissue, a noteworthy deviation from the normal being that the mucin is stainable in the cells.

These observations, it seems to me, indicate that the fat cell and the fibroblast (considering the mucous connective-tissue cell as a modified fibroblast) are very closely related and give support to the hypothesis that the fat cell is derived from the fibroblast.

REFERENCES

- (1) KNOX: Proc. New York Path. Soc., 1919, xix, 90.
- (2) HIRSCH AND WELLS: Am. J. M. Sc., 1920, clix, 359.
- (3) TODD AND BOWMAN: Physiol. Anat., 1845, i, 90.
- (4) TOLDT: Quoted by E. A. Schaafer, Textbook of Microscopic Anatomy, New York, 1912, p. 130 (Quain's Anatomy, vol. ii, pt. 1).
- (5) FLEMMING: Arch. f. mikr. Anat., 1876, xii, 434.
- (6) HAMMAR: Arch. f. mikr. Anat., 1895, xlv, 512.
- (7) BORDEN: New York M. J., 1894, lix, 225.
- (8) BELL: Am. J. Anat., 1909, ix, 401.
- (9) LEWIS AND STÖHR: Textbook of Histology, 2d ed., Philadelphia, 1917, p. 62.
- (10) MALLORY: Principles of Pathologic Histology, Philadelphia, 1914, p. 84.
- (11) EWING: Neoplastic Diseases, Philadelphia, 1919, pp. 166, 167.

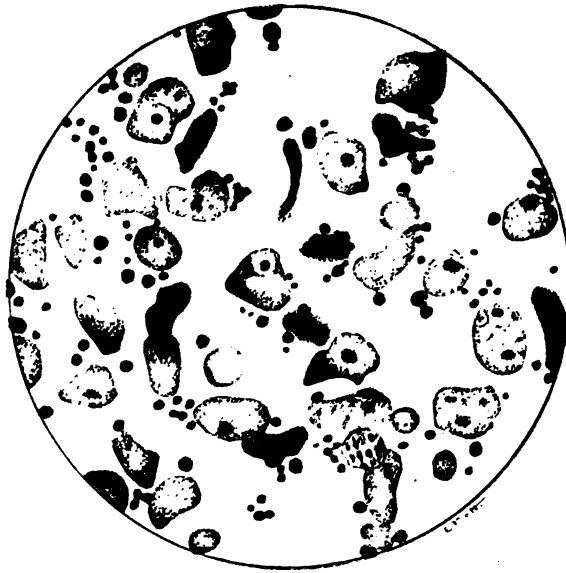
PLATE

PLATE 1

FIG. 3. Drawing of microscopical field, high power. Frozen section stained with Scharlach R and hematoxylin. The distribution of fat in the cells and interstices is shown. In the center are two cells in mitosis and containing fat droplets.

FIG. 4. Drawings of four cells in a section stained with Scharlach R and hematoxylin, showing the transition from spindle cell to the mature signet-ring fat cell.

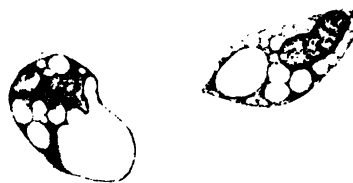
FIG. 5. Drawings of three cells in a section stained with Mayer's mucicarmine, from an area of myxomatous tissue. The finely granular mucin is seen in the cytoplasm between the fat droplets.



III



IV



V

PARABIOSIS AND TUMOR GROWTH

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The subject of tumor immunity is of considerable importance, and any approach that promises to throw light upon this as yet unsolved problem should be thoroughly investigated. The effect of parabiosis upon tumor growth has been studied by various investigators, but their results and conclusions have been far from uniform. In an attempt to clear up this matter, the experiments here reported were undertaken.

Sauerbruch and Heyde (1), in a series of experiments with parabiotic animals, noticed that the death of one animal was always followed by the death of the other in a few hours unless the live animal was cut away from its dead partner. This, they maintained, is probably due to the absorption of cadaver toxins from the dead animal. They also noticed that when iodine solution was injected into one animal, the urine of the other gave a positive iodine reaction within forty-five minutes. That corpuscular elements also could pass from the one to the other, they proved by injecting a culture of anthrax bacilli into the right animal and recovering the characteristic organisms from the heart's blood of the left. From their anatomic studies, the authors conclude that the operative wound uniting the two animals heals exactly as does that in a single animal, except that the reaction is more intense, owing probably to "the foreign body" reaction.

Friedberger and Nasseti (2) studying the antibody formation in parabiosis found that agglutinins formed as a result of the injection of typhoid bacilli or *Vibrio elbensis* into one animal could be recovered from the non-injected one. They also demonstrated that one animal could be immunized passively by the

injection of these organisms into the other. Morpurgo (3), in a series of most interesting experiments, showed convincingly that after a bilateral nephrectomy was performed upon one rat, the kidneys of its partner in parabiosis were able to compensate for their loss and maintain life for both animals—in one instance for forty days. In another case, he succeeded in keeping two parabiotic rats alive for seventeen days after unilateral nephrectomy in one animal, the two kidneys of the other having been previously removed. Jehn (4) and Sauerbruch and Heyde obtained similar results in rabbits.

The findings in all these investigations show how very intimate is the biological relationship between the parabiotic animals. Lambert (5) studied the influence of parabiosis upon the growth of transplantable tumors in rats, and concluded that the growth of mouse tumors in rats is definitely promoted by parabiosis in that the percentage of successful inoculations is increased, the rate of growth accelerated, and the duration of active growth extended. Rous (6) in a series of experiments with rats concluded that parabiosis has no effect upon the growth of tumors. Albrecht and Hecht (7), however, showed that parabiosis in mice exerted a distinct retarding influence on the growth of tumors, and explained this phenomenon as similar to the immunizing action of subcutaneous injections of blood, embryo, or normal tissue.

In a previous study (8) the author showed that the intraperitoneal injection of blood from immune animals into susceptible ones was without retarding influence upon the growth of tumors in the latter. This paper is a continuation of the previous study and is based on the following working hypothesis: We assume that an immune animal possesses substances antagonistic to tumor growth, or that it lacks those elements necessary to tumor for its growth. Having proved that these substances do not reside in the circulating blood, we further assume that they exist somewhere else in the organism. By performing a parabiosis between a refractory and a susceptible animal, we bring these two organisms into very intimate biological relationship. We have further assumed that it may be possible

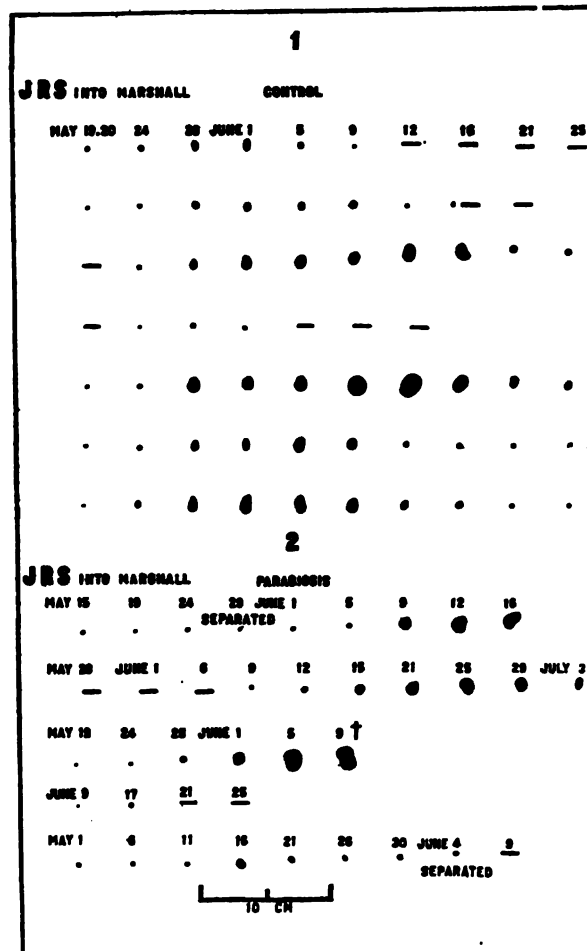
(1) for the "antibodies" in the immune animal to cross over into the non-immune parabiotic partner and confer upon it the property of antagonism to tumor growth; (2) for the substances that make for tumor development in the susceptible animal to cross over into the immune one and render that animal susceptible. To test these possibilities this study was undertaken.

In one part of these experiments, 44 pairs of animals were united in parabiosis. Two distinct breeds of animals were employed—August rats, which are susceptible to the growth of Jensen rat sarcoma (J R S) and Marshall rats which are refractory to this tumor. Five days subsequent to the uniting of two of these animals, the Marshall rat was inoculated with 0.003 gram of Jensen rat sarcoma. Of the 44 pairs, 3 died before the tumor inoculation. The parabiotic life period of the others varied from four to thirty-five days. Five pairs were lost leaving 36 pairs from which to draw conclusions. In only sixteen instances (44 per cent) could tumor formation be made out and at that the majority of these growths were pinhead size. Of the control animals, individual rats of the same breed receiving similar inoculations of Jensen rat sarcoma, 32 out of 43 (74 per cent) showed positive results. These tumors were larger, appeared earlier, and lasted longer than those in the parabiotic series.

In chart 1 are shown the tracings of the growth of the Jensen rat sarcoma in the Marshall rat. The gradual growth soon reaches its peak and in a short period of time the tumor recedes and disappears completely. In the parabiotic Marshall rats, in practically all cases where the tumor does develop, it fails to grow as well as in the controls and recedes much more rapidly. In the first two instances on chart 2 it should be noted that the rapid growth took place only after the August animal had died. The obvious conclusions are (1) that the susceptible animal does not transmit any of its characteristics to the immune one; (2) that parabiosis reduces the rate, intensity, and duration of growth of the Jensen rat sarcoma in the Marshall rat. This is probably due to the ill health of the animal in parabiosis.

In a second series, tests were made to determine whether protective substances are transmitted from the immune to the

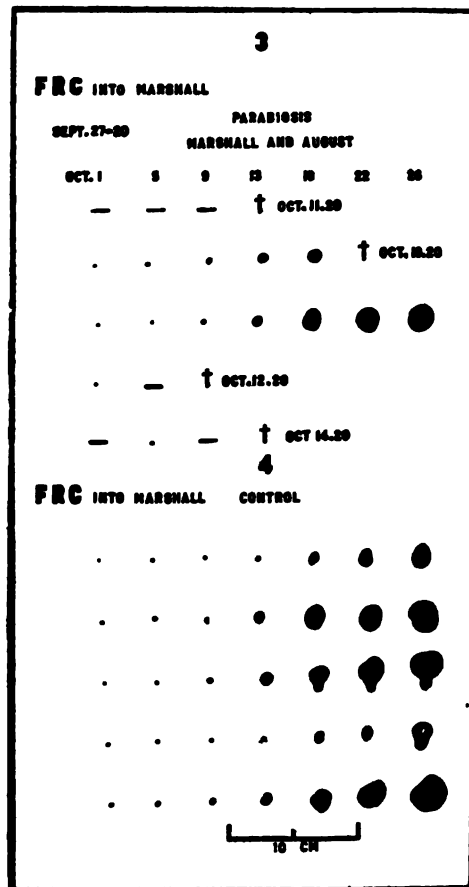
susceptible animal, in other words, whether passive immunity can be developed in this manner. The susceptible Marshall rats were inoculated with Flexner rat carcinoma (FRC). Nine



CHARTS 1 AND 2

pairs were operated upon; six pairs survived the four day post-operative period and were inoculated with the tumor. Two survived for a period of over two weeks, which allowed chartings

enough to make a fair comparison with the control. Chart 3 shows that the growths in time of appearance, and rapidity of development are only slightly behind those of the controls.



CHARTS 3 AND 4

CONCLUSIONS

From these experiments it is quite logical to conclude that parabiosis does not increase the susceptibility of the immune animal nor does it give immunity to the susceptible one, and that whatever the substances are that make for growth in the suscepti-

ble and for non-growth in the immune animal, these properties are not carried over by the parabiotic union. The lesser size of the tumor and the lesser rapidity of its growth in the susceptible partner can be explained by the setback that the operation has produced and by the interference with the normal activities of the animal, and it is unnecessary to assume as do Albrecht and Hecht that this phenomenon is one of partial artificial immunity similar to that produced by the injection of homologous tissue, since such immunity is known not to affect tumors after growth is begun but only to prevent inoculation. While the number of animals employed in these experiments is rather small, the results, taken in conjunction with those of other observers in this field, are sufficiently consistent to make it unnecessary to repeat them in a larger series.

REFERENCES

- (1) SAUERBRUCH AND HEYDE: München. med. Wehnschr., 1908, lv, 153.
- (2) FRIEDBERGER AND NASSETTI: Ztschr. f. Immunitätsforsch. u. exper. Therap., 1909, ii, 509.
- (3) MORPURGO: Verhandl. d. deutsch. path. Gesellsch., 1910, xiv, 259; 1909, xiii, 150.
- (4) JEHN: Ztschr. f. exper. Path. u. Therap., 1909, vi, 16.
- (5) LAMBERT, R. A.: J. Exper. M., 1911, xiii, 257.
- (6) ROUS: J. Exper. M., 1909, xi, 810; Proc. Soc. Exper. Biol. and Med., 1909-10, vii, 12.
- (7) ALBRECHT AND HECHT: Centralbl. f. allg. Path., 1909, xx, 1039.
- (8) KROSS: J. Cancer Research, 1921, vi, 25.

THE PROTEIN CONTENT OF THE WHOLE BLOOD AND PLASMA IN CANCER

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In 1919, Dr. William S. Stone and the writer (1) reported a study of the chemical composition of the blood in cancer cases, describing the results of determinations of the sugar and the non-protein nitrogen constituents of the blood. It has been a question for years whether the proteins of the blood are increased or decreased in cancer. Only recently Robin (2) stated that the serum protein is increased, but, like so many other investigators, he based his conclusions on the study of only a few bloods. Therefore, it seemed advisable to investigate a sufficient number of cases and compare the results with those obtained in other diseases.

The nitrogen of the whole blood, serum, or plasma was determined by the Kjeldahl method, and the hemoglobin with Dare's hemoglobinometer. Cancer bloods were obtained from the wards of the Memorial Hospital, and the others from the Roosevelt Hospital.

Forty-three bloods were examined. Tables 1 and 2 show the results obtained on cancer bloods, the only difference being that table 1 refers to serum and table 2 to plasma. Table 3 contains the results on bloods from other pathological conditions.

The protein content of the whole blood depends to such a large extent upon the amount of hemoglobin present that we should expect a large variation. Thus, in table 1 whole blood protein varies from 12 to 19 per cent, with an average of 16.4 per cent.

TABLE 1
Protein of whole blood and serum in cancer

NUMBER	DIAGNOSIS	WHOLE BLOOD PROTEIN*	SERUM PROTEIN	DARE HEMOGLOBIN
		per cent	per cent	per cent
1	Papilloma of bladder	12.1	7.7	40
2	Carcinoma of bladder	17.6	6.6	80
3	Carcinoma of bladder	16.2	6.0	90
4	Carcinoma of prostate	19.0	7.3	
5	Carcinoma of prostate	19.1	6.7	80
6	Carcinoma of prostate	12.1	6.9	55
7	Carcinoma of prostate	16.2	8.2	
8	Carcinoma of parotid gland	17.8	6.9	
9	Carcinoma of uterus	14.1	7.1	51
10	Carcinoma of rectum	18.1	6.4	90
11	Epithelioma of jaw	16.2	6.0	85
12	Epithelioma of tonsil	16.8	6.6	75
13	Neurosarcoma of arm	18.2	7.7	75

*Nitrogen \times 6.25 = protein.

TABLE 2
Protein of whole blood and plasma in cancer

NUMBER	DIAGNOSIS	WHOLE BLOOD PROTEIN*	PLASMA PROTEIN	DARE HEMOGLOBIN
		per cent	per cent	per cent
14	Carcinoma of bladder	16.9	7.1	75
15	Carcinoma of bladder	13.2	5.3	60
16	Carcinoma of bladder	16.1	5.6	
17	Carcinoma of bladder	18.8	7.1	
18	Carcinoma of uterus	15.5	5.6	60
19	Carcinoma of breast	14.7	6.1	75
20	Carcinoma of breast	15.7	5.9	
21	Carcinoma of rectum	14.4	8.1	
22	Carcinoma of rectum	15.0	5.9	
23	Carcinoma of liver	15.8	6.1	
24	Carcinoma of larynx	14.7	6.7	
25	Carcinoma of stomach	8.7	5.1	
26	Teratoma of testis	18.2	6.7	
27	Keloid of chest	19.3	7.4	

*Nitrogen \times 6.25 = protein.

In table 2 the protein varies from 13 to 19 per cent with an average of 16.3 per cent, if we except case no. 25, in which the blood was very anemic with a protein value of only 8.7 per cent.

In table 3 the protein varies from 12 to 20 per cent with an average of 17.6 per cent. Hemoglobin readings were, unfortunately, not done in these cases but they would undoubtedly have averaged higher than in the cancer cases and would account for the slightly higher protein value of the whole blood.

If there is an increase or decrease in the amount of protein in cancer blood, it would show in the serum or plasma. Here we can see that the variation is very slight. Table 1 varies from 5.9 to 8.1 per cent with an average of 6.8 per cent; the patient

TABLE 3
Protein of whole blood and plasma in other diseases

NUMBER	DIAGNOSIS	WHOLE BLOOD PROTEIN*	PLASMA PROTEIN
		per cent	per cent
1	Hematoma of kidney	19.8	7.2
2	Tuberculous glands	16.4	5.4
3	Colon bacillus infection	18.2	5.8
4	Paralysis agitans	19.6	7.9
5	Pleurisy	15.3	6.0
6	Ear infection	19.6	8.0
7	Retroversion of uterus	16.0	6.9
8	Urticaria	17.9	8.1
9	Possible adhesions	19.6	7.6
10	Neurasthenia	17.8	7.2
11	Neurasthenia	17.3	7.4
12	Pyelitis	18.4	8.0
13	Pyelitis	12.3	7.9
14	Pyelitis	20.0	5.9
15	Pyelitis	18.4	7.2
16	Pyelitis	16.3	8.0

*Nitrogen \times 6.25 = protein.

whose blood had the highest value died the day after the blood was taken. The plasma protein values of table 2 varied from 5 to 8.1 per cent, with an average of 6.3 per cent. The plasma values of other pathological conditions varied from 5.3 to 8 per cent, with an average of 7.0 per cent.

Loebner (3) reported results on 44 cases of cancer. She employed the refractometric method, using serum obtained from finger blood. Her figures varied from 4.2 per cent to 9 per cent with an average of 7.3 per cent, which corresponds very closely

with the average of this series. She considered 8.2 per cent the average for normals since that is the average which Heudorfer obtained in a study of five cases. This would indicate that the protein of the serum is somewhat reduced in cancer. If, however, we compare the average in cancer cases with the average in cases of other pathological conditions, we can see that there really is no difference in the amount of protein present.

CONCLUSION

Proteins of the blood plasma are neither decreased nor increased in cancer cases as compared with other hospital patients.

REFERENCES

- (1) THEIS AND STONE: Jour. Cancer Res., 1919, iv, 349.
- (2) ROBIN: Bull. de l'Acad. de méd., 1920, lxxxiv, 51.
- (3) LOEBNER: Deutsch. Arch. f. klin. Med., 1918, cxxvi, 61.

PROBLEMS IN CANCER RESEARCH¹

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It is a pleasure to announce that a Department for Cancer Research has again been established in St. Louis by the Barnard Free Skin and Cancer Hospital. The material available is the laboratories and what can be developed from the more general biological side within them, and the clinical material.

The plan which is being used for the study of cancer divides the work into several distinct groups. One is a general study of the properties of the various cells of the body and the peculiarities of the mechanisms of growth, division, differentiation, and function of these cells. A second is a close study of the clinical course of the disease and the results of methods of treatment and diagnosis. A third is an attempted analysis of isolated facts now fully demonstrated, and their immediate relation to clinical and pathological data at hand.

For the immediate time, one problem which seems to be most pressing is the improvement of methods of diagnosis, especially the diagnosis of internal cancer. Early diagnosis of cancer of the breast has prolonged the life of many patients for at least ten or twenty years. The same is true for other superficial growths. Barring a few exceptional cases, methods for the diagnosis of internal cancer have not reached the same degree of perfection, as, for instance, cancers of the intestinal canal. If by chance a cancer of the intestine causes early obstruction, a diagnosis may be made and the treatment may be hopeful. How few cancers of this region give, however, this early symptom!

¹ Presented before the American Association for Cancer Research, Cleveland, Ohio, March 24, 1921.

Another of the immediate problems is the betterment of methods of treatment now available. The third and most important of all is the etiology of this disease. It is most important, because through its elucidation and even in its study all other problems must become simplified. Through it alone, barring some peculiar accident, can the hopes for ultimate success in treatment come. The greatest effort in cancer must be exerted, therefore, in this direction.

What is known about cancer today, aside from clinical considerations, is little more than was known many years ago. This is appalling but true, and the question arises: Is it not due to the fact that the general notions of the cell, its structure and properties have not materially advanced during this time? The problem of the etiology of cancer when resolved into terms of the cell may be quoted thus: Is the cancer cell a cell different from normal cells, or is it a normal cell suffering a continuous external growth stimulus? Since the application of the theories of cellular growth now in vogue have failed to answer this question, there remain open but two channels of attack; the hit and miss method, and a further probing into the nature of the mechanisms of cellular growth, division, differentiation, and function.

Cancer, no matter how it is taken, represents a break in the normal growth-regulating conditions of the body. Without a knowledge of the normal growth-regulating mechanism, it is to be expected that the etiology of cancer will remain obscure, unless it is proved that the disease is the result of some definite foreign agent or stimulus—parasite, or otherwise—which may be isolated.

It is known that the body is composed of cells. The minute metabolism of these cells, the mechanism of their formation, even the simplest constituents of their life are, however, based only on theories. The leucocytes are compared to the amoeba. One talks glibly of cellular metabolism, when he probably means body metabolism. Is this so-called cellular metabolism the work of the cell or is it the work of the organ or the body as a whole? In beginning a few years ago the study of the cells in tissue culture, it was of interest to note that the leucocytes, both the various mononuclear as well as the polymorphonuclear types, show

no ability to grow and divide like other cells. Their movements are passive reactions to external conditions. A connective tissue cell or a heart muscle cell suffering from the effects of certain toxic poisons can be made to resemble the small and large mononuclear cells of the inflammatory exudate. These latter cells have lost, also, all ability for further growth under the conditions in which they had previously grown or under any other conditions it has been possible to impose upon them. In actively growing culture many of these fixed tissue cells, unaffected by foreign poisons may also show such changes after they have been preyed upon by actively growing cells.

The polymorphonuclear leucocyte is a cell differentiated in a special locality. Lymphocytes undoubtedly are found in the same locality as well as in lymph-nodes. Whether the large number of mononuclear cells of the chronic inflammatory area or about cancers have this origin is still a very much open question. In fact, the work of Maximow has not been confirmed. (See Stockard (1).) That these cells contain enzymes and bacteriolytic substances which are liberated when they break down is, again, well established. What is not known are the conditions under which these cells live in the areas about a cancer and whether they do liberate these substances under ordinary conditions there. It seems evident, therefore, that they might as well be considered the result of active growth or the presence of toxic substances, as to be thought of as antagonists to these conditions. In chronic inflammations, it is generally where the bacteria are most active that they are most prevalent. In the tissue culture it is the actively growing tissue cells that show the true antagonistic action to growing bacteria. The lymphocytic cells and the rounded-off connective tissue cells intermingle in the cultures with the bacteria. The connective tissue cells, the heart muscle cells, and other actively growing cells cease to grow when they come within the zone of bacterial action. The bacteria stand them off as they also stand off the bacteria (2).

This absence of an absolute knowledge of many of the most important functions of body cells must in itself make it very unsafe, therefore, to draw any far reaching functional conclusions

from morphological data. It makes it absolutely necessary to draw one's conclusions from the clinical facts alone. This applies not alone to treatment, but also to other experimental results. The laboratory in Saint Louis is well situated in this regard in that it is a definite part of the clinic, and also allows the broader type of biological research which in the light of present knowledge must form an intimate part of the cancer laboratory.

PROBLEMS UNDER CONSIDERATION

1. Studies on oxidation in actively growing and differentiated cells, especially in reference to their growth

In a previous communication (3) before this society evidence was given by the author to show that connective tissue cells and simple mesenchymatous and embryonic muscle cells show no evidence of an internal organization suitable for growth. They act more like homogeneous fluid systems, leaving aside the nucleus and the astral center. Growth in them can be explained entirely as a surface tension phenomenon, the energy of the process being effected by the formation of a substance within the cell, which is insoluble in circulating body fluid, but soluble in dead cells, fibrin, and products of disintegrating cells. A cell forming this substance and brought in contact with fibrin suffers a decrease in surface tension along the line of contact. The further details of the organization peculiar to growth is determined by these contacts. The order of the contacts of a growing and dividing heart muscle cell is different from a rhythmically contracting one.

For a long time it has been known that actively growing tissue cells, such as those taken from young embryos and sarcomata commence their growth in the culture within one or two hours or much earlier than adult tissues. This is seen in a study of tissues of embryo chicks. The cells from a fragment of the heart of a two, three, or four days old chick embryo will commence to grow almost at once when placed in the culture. About fragments of a ten day old embryo heart such activity is not observed until after six or twelve hours, and about frag-

ments of a fifteen day old chick embryo heart after fifteen or twenty hours; while about young adult heart fragments this latent period is often as long as twenty-four or forty hours.

In a previous publication (4), the writer reported a study of the relation of oxygen to these changes in the cell. A method was developed which allowed accurate regulation of the amount of this gas in the chamber about the cells. The tissues especially studied were fragments of the heart and body wall of fourteen, fifteen, and sixteen days old chick embryos. It was found that the growth was as active in an atmosphere of 9 per cent oxygen as in pure oxygen, and ceased in atmospheres which contained slightly less than 6 per cent of this gas. It became of interest this year to see whether the more actively growing tissue might not show variations in these figures. The question that arose was whether the less actively growing tissues did not have to form or in some other way liberate the substance or substances which actively change surface tension or produce energy. The short latent period in the more actively growing cells suggested a supercharging of this substance or substances.

In 1903, Fletcher (5) showed that a muscle of the adult frog will give maximum contractions every five minutes for a period of two hours in an atmosphere of pure nitrogen. In the work on organization of isolated rhythmical, contracting heart muscle cells in the tissue culture, the author has found evidence to show that the chemical changes of muscular contraction are similar in certain stages to those of growth. If muscle will contract for a time without oxygen the question that arises is: Will not cells fully differentiated for growth show the same reaction (6)?

The tissue so far tested has been cells from the hearts of chick embryos of various ages, ranging from four to fifteen days. Fletcher's experiments were repeated with contracting hearts and heart muscle fragments of these embryos isolated in the tissue culture. These cells will contract for from twenty to twenty-two hours in an atmosphere of nitrogen. In the same oxygen-free atmosphere, the cells from fragments of four and five days old chick embryos will grow from four to six hours. This

growth commences, however, always after a considerably longer latent period than that in an atmosphere containing oxygen. It is active for this short time, after which the cells invariably disintegrate rapidly. The period of growth in these hanging-drop cultures is very much longer in air; the early disintegration is rarely or never seen; and when it occurs, it is always later. To obtain a similar growth about the fragments of the heart of ten days old chick embryos 1.8 per cent oxygen was required and about fragments of fifteen days old chick embryos 4.5 per cent.

These experiments have not only been applicable to the problem in question, but have given a method of differentiating functionally the actively growing young embryonic cells from those showing greater degrees of differentiation. In the case of these cells they have also shown the necessity of oxygen for the maintenance of structure. Whether this method may be used for differentiating any actively growing tissue is a problem yet to be considered.

STUDIES OF PLANT CANCERS

Another method which has appealed to us in attacking the cancer problem has been the study of plant cancers. These represent an active proliferative growth, the etiology of which is known. Chambers (7) has undertaken this problem. He was interested during last summer in investigating the metabolic activity of the organism, *Bacterium tumefaciens*, in culture, and in the course of this study noted that these organisms produce alkali in the culture even in the presence of sugar, which they also cause to disappear rapidly. Smith, by a different method, had noted that ammonia is formed in the cultures of these organisms. Harvey (8) more recently, also, noted that the tissue of these tumors is more alkaline than normal plant tissue. The work of Chambers would indicate, therefore, that the increase in the OH-ion in the tissue of these tumors is due to the etiological agent, which produces alkali in the cultures at least. Chambers is now attempting to see if under the condition imposed on them by the plant, they also form it.

These facts attracted our attention to the work of Menten (9), by which an alkalosis was demonstrated in the blood of cancer patients; and this question is now under investigation. The various methods of testing were critically reviewed and the indicator method was selected. The tests are made on a dialysate of venous blood, which is taken under oil and kept unexposed to air throughout the whole procedure. The results so far obtained have been interesting. In the early part of the disease, these patients show no evidence of change in the reaction of their blood when it is taken from a region far removed from the cancer. A definite alkalosis is observed only when the cancer has grown to considerable extent and involves neighboring glands and tissues. The blood of a patient with a beginning carcinoma in the lip is normal. A definite H-ion change in the blood is not seen until the glands of the neck are involved, and then increases gradually as the disease progresses. The same is true for carcinoma of the intestine. A small annular carcinoma of the intestine with no or but little glandular involvement may show by the indicator method no change in the alkalinity of the blood. Sharp changes are always seen, however, when liver nodules are present. The CO_2 relations in the blood of these patients is now being studied. It is possible that by this means much concerning the significance of this alkalosis may be determined. The anemia may also have an effect.

The importance of these experiments is still to be ascertained. That the alkali is probably produced in the malignant tumor is indicated, however, in the study of one patient suffering from a sarcoma of the forearm. Blood taken directly from a vein leading from the tumor showed a pH of 7.5, while in the other arm the blood pH was 7.3, or normal. This case illustrates the difficulty of using such a method for a clinical test. The body compensates more readily for acid than alkali, but at the same time there is a rapid elimination of alkali by the kidney. In the case of acid, the compensating mechanism gives evidence. To what extent this can be used for determining the presence of alkali is yet to be determined. In the case cited, there was evidently alkali being given into the blood by the tumor, but it

was not detectable in the other arm. There was no glandular involvement in this case.

What the further studies of this reaction will open up and the further studies of the bacteria of the plant tumors may reveal is a question which the future alone can answer. It seems, however, that this method may yield something important.

REFERENCES

- (1) STOCKARD, R.: An experimental analysis of the origin and relationship of blood corpuscles and the lining cells of vessels. *Proc. Nat. Acad. of Sc.*, 1915, i, 556.
- (2) BURROWS, M. T.: Unpublished notes.
- (3) BURROWS, M. T.: The tissue culture in cancer. *Proc. Second Pan American Sc. Cong.*, 1915-1916, Sec. viii. Pt. 2, pp. 494-496.
- (4) BURROWS, M. T.: The oxygen pressure necessary for tissue activity. *Am. J. Physiol.*, 1917, xliii, 13-21.
- (5) FLETCHER, W. M.: The influence of oxygen upon the survival respiration of muscle. *J. Physiol.*, 1917, xxviii, 354-359.
- (6) BURROWS, M. T.: The reserve energy of actively growing tissues. *Proc. Soc. Exper. Biol. and Med.*, 1921, xviii, 133.
- (7) CHAMBERS, W. H.: Paper before the Am. Physiol. Soc., Chicago, December, 1920.
- (8) HAEVEY, R. B.: Relation of catalase, oxydase and H-ion concentration to the formation of overgrowths. *Am. J. Botany*, 1920, vii, 211-221.
- (9) MENTEN, MAUD L.: The alkalinity of the blood in malignancy and other pathological conditions; together with observations on the relation of the alkalinity of the blood to barometric pressure. *J. Cancer Res.*, 1917, ii, 179-211.

THE INFLUENCE OF HEREDITY IN DETERMINING TUMOR METASTASES¹

STUDIES IN THE INCIDENCE AND INHERITABILITY OF SPONTANEOUS TUMORS IN MICE

SIXTEENTH REPORT

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Tumor metastases in this stock are somewhat rare. In 29,000 necropsies, furnishing something over 4000 primary spontaneous tumors, mostly malignant, only about 19 per cent of the tumors have metastasized. It has been the general opinion of pathologists that the formation of metastases is a distinguishing property of malignant tumors. The infiltration of tumor cells into tissues adjacent to the neoplasm, and the entrance of these cells into the lymph and the blood streams are the usual mechanical bases for secondary growths. But all these conditions exist in innumerable instances, both in human and in animal tumors, without the occurrence of secondary growths. For example, females 7821 and 9260 of this stock (see chart 11, strain 508, p. 158) each had a large and highly malignant infiltrative mammary gland carcinoma of relatively long standing. In both instances there were multiple tumor emboli in the lungs, but no secondary growths arose from these emboli,—i.e., the mechanical basis was there in each case, the occurrence of tumor did not follow.

¹ Presented before the American Association for Cancer Research, Cleveland, Ohio, March 24, 1921.

Ewing (1), in his chapter on metastasis, speaks of "a peculiar susceptibility of some tissues to develop secondary tumors," but later states that "the mechanism of circulation will doubtless explain most of these peculiarities, for as yet there is no evidence that any one parenchymatous organ is more adapted than others to the growth of embolic tumor cells." There would seem, then, to be some factor, other than the mechanical one, which determines the incidence and location of metastasis, even though it be minimized, as Ewing has done in the paragraph cited.

Again, Ewing states that in highly vascular tissues like the lip, stomach, and testicle, very small carcinoma may yield distant metastases. But these very organs are among those whose tumors rarely metastasize in this stock. For example, among the lip carcinomas in this stock none has metastasized; among the testicle tumors (2), one metastasized locally, while five out of six stomach carcinomas (3), (4), showed metastases in regional lymph-nodes only, but no vascular metastases in the liver or elsewhere. And this in spite of the close resemblance of these tumors to human tumors of similar type in similar organs.

In this stock, mammary gland carcinoma and sarcoma rarely metastasize into regional lymph-nodes, in marked contrast to human breast tumors of the same type. Indeed, in this stock, mammary gland tumors can be seen growing up to the lymph-node but not invading it, even by extension. On the other hand, pulmonary metastases from mammary gland carcinoma and sarcoma are common in this stock, again in contradistinction to the reported behavior of human breast tumors of the same types.

These divergencies (which are typical of the many divergencies) in the metastasis behavior of tumors of similar types and in similar organs, require some explanation other than merely the mechanical tendency of certain types of tumors to metastasize in certain locations, and that there is some other factor is generally recognized by pathologists, although little progress has as yet been made in demonstrating what this factor is.

The matter of tumor metastasis in this laboratory has been under observation for twelve years, and the data here given are based on over 29,000 necropsies, among which every instance of metastasis is known. The charts used to demonstrate the points made in this report are perfectly typical. The non-tumor fraternities (where there are any) have been omitted, in order to save space and to show exactly where these tumors occurred.

It must be borne in mind in reading these charts, that, as I have previously stated, tumor metastases in this stock are rather rare, being only about 19 per cent of the primary malignant tumors. A given chart then, which shows many primary tumors, must not be expected to show large numbers of secondary tumors. For example, a chart showing 8 primary tumors, would, if this general average of 19 per cent were carried out, show only 1.5 cases of metastasis, etc. The biological evidence from this laboratory, which explains this low percentage of metastasis from malignant tumors, will be given further on in this report.

From this study three points stand out with striking clearness:

POINT I

In any given strain the metastatic tumors (where there are any) tend to occur in exactly the same organs in which the primary tumors of that strain occur.

Note chart 1, showing part of strain 215 and some of its derivatives. The parent female, no. 3, had a sarcoma-carcinoma of the mammary gland, a malignant adenoma of the liver, and a metastatic sarcoma of the kidney. The parent male, no. 360, came from a strain carrying lung and mediastinal tumors, and he himself was proved heterozygous to tumors in these locations (i.e., he carried them potentially and introduced them in every strain into which he was crossed, although he did not himself show these tumors).

Note here how these particular types and locations of tumors have "segregated out" and certain of them have been transmitted to the branches of the strain here shown, both as primary

and as secondary neoplasms. In the first hybrid generation, male 1192 had a primary carcinoma of the lung and a secondary carcinoma of the lung. Female 5 had a primary carcinoma of the mammary gland, with multiple secondaries in the lung. Note how the different types and locations of tumors have "segregated out," so that in branch A lung tumors alone have been transmitted, and a 100 per cent lung tumor strain is being extracted. In branch B liver tumors have predominantly been transmitted, from female 630 with a secondary tumor in the liver, so that in one part of the branch a 50 per cent liver tumor strain is being extracted.

In these two branches of the strain, then, female 3 introduced:

a. Primary carcinoma of the mammary gland, in females 5, 2239, and 630.

b. Primary adenoma of the liver, in females 1681, 8871, 11237, and 12207, and in male 2327. (Note the three consecutive generations of adenoma of the liver.)

c. Secondary carcinoma of the liver, in female 630.

d. One case of sarcoma, in female 5543.

Parent male 360 introduced:

a. Primary carcinoma and adenoma of the lung, in males 1192, and 900, and in females 326, 816, 823, 3191, 2548, and 6688.

b. Secondary carcinoma of the lung, in male 1192 and in females 5 and 630.

c. Primary tumor of the mediastinum, in female 5543.

d. Secondary tumor of the mediastinum, in female 326.

Note how female 5543, with a lymphosarcoma of the mediastinum, derived the type of her tumor from her maternal ancestor (female 3) and the location of her tumor from the paternal ancestor, male 360 (who carried these tumors potentially, and transmitted them in every strain into which he was crossed, although he himself did not develop them), just as in the human species, a child might inherit curly red hair from a mother with curly black hair, and a father with straight red hair.

Note how female 5 derived her primary tumor from the maternal ancestor, and her secondary tumor from the paternal ancestor, and how female 630 derived her primary tumor from

the parent female, the secondary in the lungs from the parent male, and the secondary tumor of the liver from the parent female—a marked demonstration of the “segregating out” of these things, which is the *heredity test*. Note how, after the second hybrid generation, no further mammary gland tumors occurred in either of these two branches.

Note that there occur no tumors, either primary or secondary, which were not introduced by the parents. Note that the secondary tumors occur in the same organs as the primary tumors.

Chart 2 shows parts of strains 48 and 292. These are two strains derived from the same original mating as was strain 215, that is, female 3 (with a sarcoma-carcinoma of the mammary gland, a malignant adenoma of the liver, and a metastatic sarcoma of the kidney), and male 360 who introduced carcinoma of the lung and the mediastinum. Here again the particular types and locations of tumors introduced by the parents have “segregated out” and been transmitted to the resulting strains, both as primary and as secondary neoplasms.

In the first hybrid generation female 5 derived from the mother a carcinoma of the mammary gland, and from the father a secondary carcinoma of the lung. In the second hybrid generation, female 37 derived from the parent male a carcinoma of the lung, with a secondary carcinoma in the mediastinum.

In the third and fourth hybrid generations, note the outcropping of sarcoma, both primary and secondary, in females 1454, 26, 348 and 396. Liver tumors derived from female 3, also occur in these strains, as shown in females 5743, and 26, and in male 11836. There is also a “pre-cancerous” liver in female 399. Here also lung tumors, derived from male 360, occur primary in females 37 and 399 and in male 11836; and secondary in females 5, 5743, and 396.

Note the occurrence of secondary *carcinoma* of the mediastinum in female 37, of secondary *sarcoma* of the mediastinum in female 396; of secondary *carcinoma* of the lung in female 5, and of secondary *sarcoma* of the lung in females 5743 and 396. Note the three cases of secondary sarcoma in the spleen in females

5743, 348, and 396. Rarely in this stock, except in strains derived from female 3, have there been any tumors of the spleen either primary or secondary.

Here again, after the first hybrid generation, mammary gland tumors disappear—another indication of how these things “segregate out.” The mammary gland tumors preponderated in other strains derived from the same original mating. The dominating tumors, both primary and secondary, in these strains are in the liver, kidney, spleen, mesentery, lungs, and mediastinum.

In these strains, then, female 3 introduced:

- a. Primary carcinoma of the mammary gland, in female 5.
- b. Primary adenoma of the liver, in female 399 and in male 11836.
- c. Primary sarcoma of the liver, in females 5743 and 26.
- d. Primary sarcoma of the kidneys, in females 26, 348, and 396.
- e. Secondary sarcoma of the kidneys, in female 1454.
- f. Secondary sarcoma of the spleen, in females 5743, 348, and 396.
- g. Primary sarcoma of the mesentery, in female 1454.
- h. Secondary sarcoma of the mesentery, in females 1454, 5743, and 26.

Parent male 360 introduced:

- a. Primary lung tumors, malignant and not yet malignant, in females 37 and 399, and in male 11836.
- b. Secondary lung tumors, in females 5743 and 396.
- c. Secondary carcinoma of the mediastinum, in female 37.
- d. Secondary sarcoma of the mediastinum, in female 396.

Note here again, how, though they are much fewer, the secondary tumors arise in the same organs as do the primary.

Chart 3 shows a part of strain 65, branch 2. The parent female, 5738, had three carcinomas of the mammary gland and metastatic tumors in the lungs. She was mated with her brother, male 782, who died of uncertain causes, but who was proved heterozygous to tumors of the mammary gland (he carried them potentially and transmitted them, but did not himself develop them). Note here that with one exception

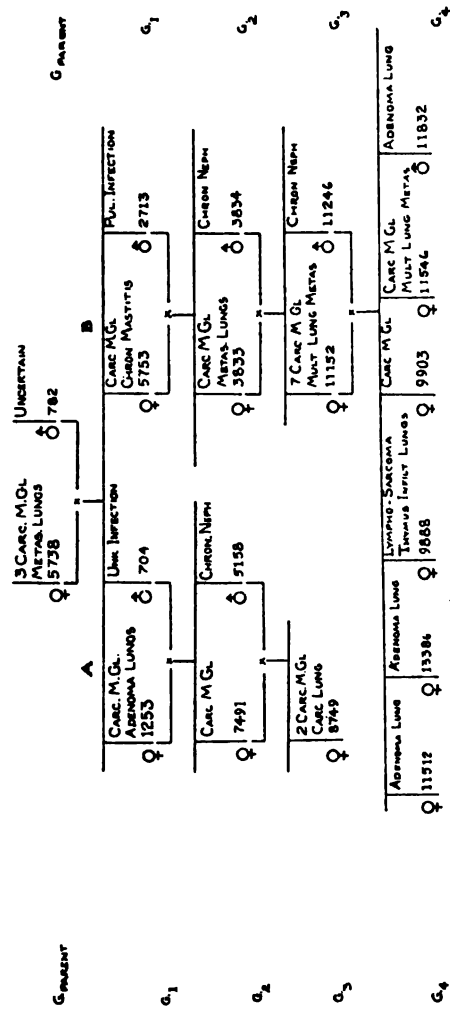
STRAIN 65 - BRIX

CHART 3

(female 9888 who had a lymphosarcoma of the thymus) the only tumors which occurred were carcinoma of the mammary gland and lung tumors, primary and secondary.

Note the five consecutive generations of carcinoma of the mammary gland in branch B, all but females 5753 and 9903 showing lung metastases. Note the heavy percentage of lung tumors primary and secondary in both branches of the strain. Note the negative evidence of the "segregating out" of the different types and locations of tumors, both primary and secondary, in the complete absence from this strain of all tumors of the liver, kidney, mesentery, spleen, pancreas, uterus, etc.

Chart 4 shows a part of strain 338, branch V, A, with partial ancestry. The parent female 3920 had an adenoma of the liver. Note the predominance of primary liver adenoma in this strain, 7 cases (over 43 per cent) and the occurrence of two cases of secondary liver tumor in the same strain, that is, female 8865 with an osteoid sarcoma of the mammary gland, secondary in the liver, and her grandson, male 16370 with an osteosarcoma of the leg, metastasizing in the liver, and also a mesothelioma of the testicle. (Testicle tumors also occur in this strain.)

Chart 5 shows a continuation of the same strain, strain 338, branch V, A (the data being too extensive for a single chart). Chart 5 begins with generation 3 of the family, the mating being between female 8619 and male 8751. Generations 7, 8, and 9 are added to the family in this chart. Note here, also, the further occurrence of liver tumors, there being one additional primary, in female 30469, who showed a primary liver adenoma in addition to seven carcinomas of the mammary gland, a primary lung adenoma, a typical uterine fibroid, and a diffuse endothelial hyperplasia of the glands and spleen. (Needless to say, the mouse was riddled with tumor, little but neoplasm remained.) There were also in this part of the family, two additional cases of secondary liver tumor, *one carcinoma* and *one sarcoma*, that is, female 22263 with three carcinomas of the mammary gland and a sarcoma in the peritoneum near the stomach, with *sarcoma metastases* in the liver, pancreas, and uterine wall, and female 30501 of generation 9, with two carci-

PART OF STRAIN 358 - BREX A - WITH PART OF ANCESTRY

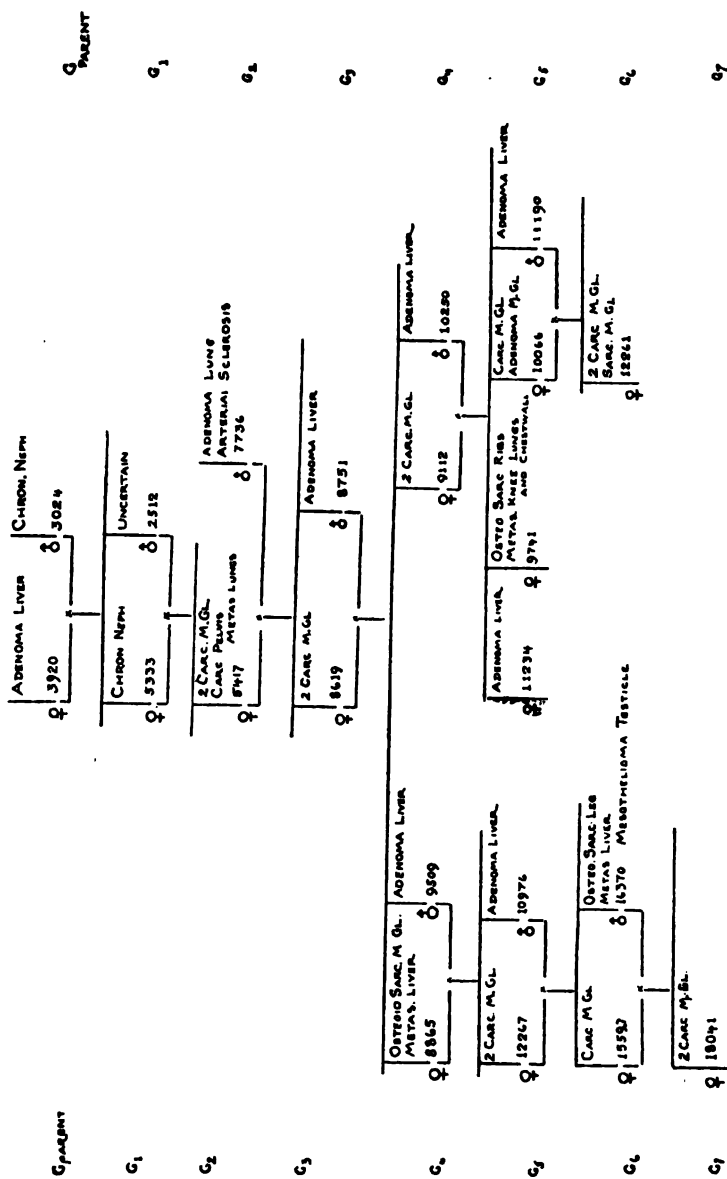


CHART 4

nomas of the mammary gland and the liver absolutely riddled with *carcinoma secondaries*.

It is interesting to note here also the two occurrences of uterine neoplasm, *primary* in female 30469, and *secondary* in female 22263, as this is one of the strains carrying most of the uterine tumors in this stock.

CONTINUATION OF STRAIN 538—BRYAN

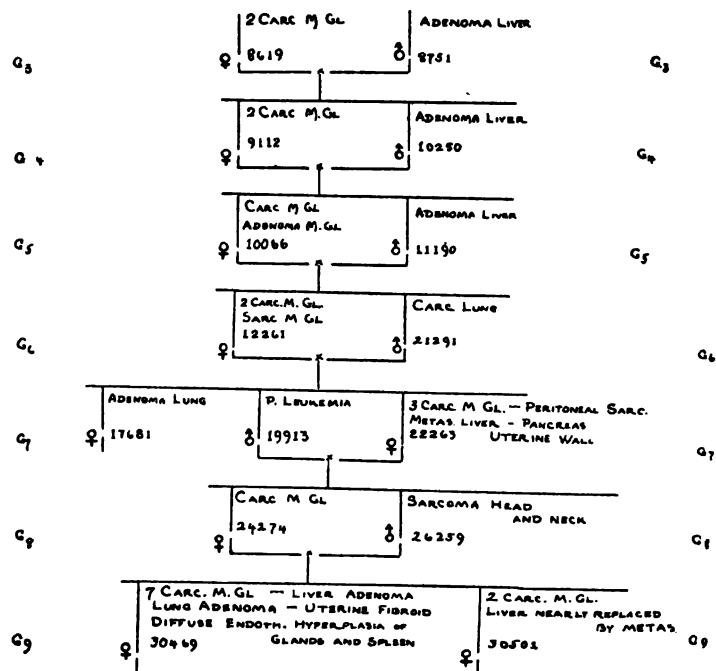


CHART 5

The pancreatic secondary sarcoma also is extremely interesting, as this is the strain which carries the only other pancreatic neoplasms which have occurred in this stock.

The complete ancestry of this strain is shown in charts 6 and 7, chart 6 being the ancestry of parent female 3920, and chart 7 the ancestry of parent male 3024. The prominence of liver tumors, primary and secondary, in the resulting strain is due to the fact that it was bred in from both sides of the family; the

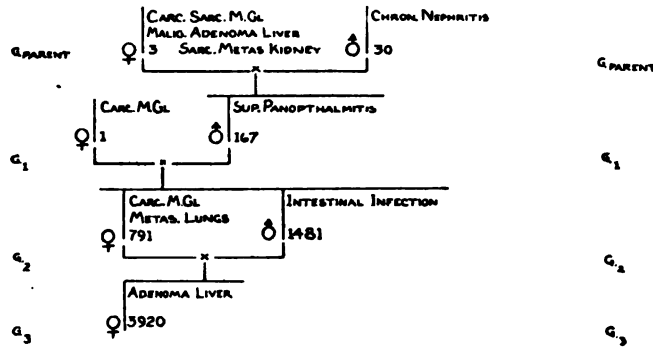
ANCESTRY OF ♀ 3920 — STRAIN 338

CHART 6

strain thus received "a double dose." Note also the marked outcropping of sarcoma in strain 338, derived from original parent female 3. Sarcoma also was bred in from both sides of the ancestry.

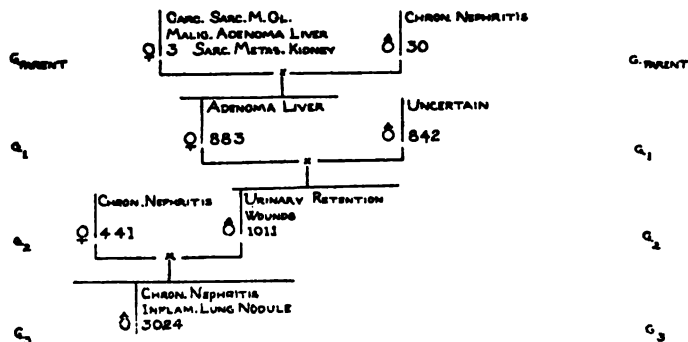
ANCESTRY OF ♂ 3024 — STRAIN 358

CHART 7

Chart 8 shows a part of strain 465, which was derived from the same original ancestry as strain 338 (ancestry shown in charts 6 and 7). Note here also, the same appearance of liver tumors, two primary and one secondary. This gives us, then,

in these two small strains (338, branch V, A and 465) derived from identical original ancestry, 10 primary and 5 secondary liver tumors. Including the ancestry, the number is 13 primary and 5 secondary liver tumors. This is a very high occurrence of liver tumors, both primary and secondary, especially when one considers the fact that outside of this stock there is just one mouse liver tumor on record, a primary tumor of the liver, re-

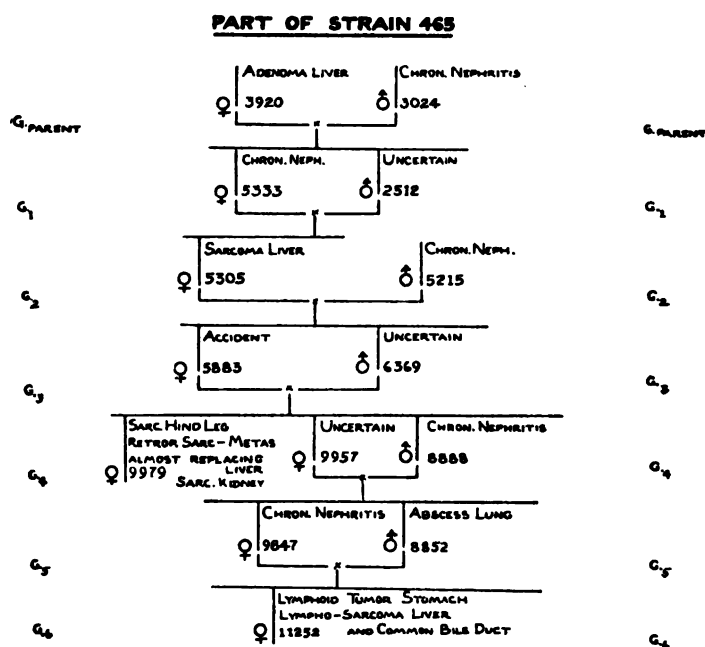


CHART 8

ported by the Imperial Cancer Research Fund of England. This liver tumor occurrence (in the Slys stock) within a single strain, makes a piece of evidence which is conclusive.

These five strains, represented in charts 1 to 8 inclusive (which, it must be remembered, are perfectly typical) furnish both *positive* and *negative* evidence of the "segregating out" of different types and locations of tumors, and their transmission as such, whether as primary or as secondary neoplasms. They

furnish, therefore, both positive and negative evidence that *in any given strain the metastatic tumors (where there are any) tend to occur in exactly the same organs in which the primary tumors of that strain occur.* Note, therefore, that:

1. Every secondary tumor of the liver in this stock (with the possible exception of two osteosarcomas, which metastasized into nearly every organ in the body, in two mice whose strains have not yet been completely analyzed) has occurred in strains carrying primary liver tumors.

2. Every secondary tumor of the kidney in this stock has occurred in strains carrying primary kidney tumors.

3. Every secondary lung tumor in this stock has occurred in strains carrying primary lung tumors (with the possible exception of the one lung metastasis in strain 164, branch IV, line C).

4. Every secondary tumor of the mediastinum in this stock has occurred in strains yielding primary tumors of the mediastinum.

5. Every secondary tumor of the pancreas in this stock has occurred in the strain which yields primary pancreatic tumors.

6. Every secondary tumor of the spleen in this stock, has occurred in strains carrying primary spleen tumors.

7. Every secondary uterine tumor in this stock has occurred in strains carrying primary uterine tumors.

When this stock, or any other, has been completely analyzed, there is little doubt that it will show that every secondary tumor of any organ whatever has arisen in a member of a strain which has shown, or will show, some cases of primary tumors in that organ (however few they may be). There is little doubt that strain 164, branch IV, line C, of this stock will in time yield a primary lung tumor, although it has not done so yet. There is a very slight inheritance of lung tumor neoplasm tendency in this line of the strain, and it is therefore to be expected that lung tumors of any kind will occur but seldom.

The complete analysis of any strain is a very long and difficult process, and the apparent testimony of the frequent occurrence of secondaries in man in tissues where primary tumors rarely occur is of no help here, as no human strain has ever been even partially analyzed.

Female 3 is listed in these charts as having a sarcoma-carcinoma of the mammary gland. This tumor was unquestionable carcinoma in some parts and apparently sarcoma in other parts. There has been considerable difference of opinion concerning these mixed tumors. Slye, Holmes, and Wells (5) Loeb (6), Lewin (7), Bashford (8), Woglom (9), and some others, diagnose them as such, while Le Count (10) and Ewing (11), etc., consider them to be entirely carcinoma, with pressure distortion of the cells simulating sarcoma in some areas.

The biological evidence of the work in this laboratory unquestionably supports the opinion that there are these mixed tumors. Female 3 with such a tumor, has unquestionably transmitted carcinoma in every strain into which she has been hybridized; and she has equally certainly transmitted sarcoma in some branch of every strain into which she has been crossed (see charts 1, 2, 4, 5 [female 3 shown in ancestry charts 6 and 7], 8 (same parentage charts 6 and 7), Chart 9).

I have preferred the term sarcoma-carcinoma to represent this type of tumor, because according to the biological evidence, it is not a sarcomatous carcinoma, nor a carcinomatous sarcoma, but rather a sarcoma plus a carcinoma, each type of tumor being transmitted separately, as such. Biological evidence, as manifested in heredity behavior, is too fundamental to be ignored.

POINT II

In certain strains there is a tendency for primary tumors to metastasize in certain organs; whereas, in other strains, tumors of the same type, primary in the same organ, even when they are of older and of larger growth, fail to metastasize into those organs.

Chart 9 shows part of strain 73, which was derived from the same female 3, mated this time with male 30. Male 30 came from a strain carrying tumors of the lung, mediastinum, and diaphragm, and was proved heterozygous to tumors of these organs, having been tested in various crosses.

The son of this mating, Jap. W. F. male, died before autopsies were made, and consequently the cause of his death is not known.

He was crossed with a Lathrop silver-faun female, who also died before autopsies were made. Their offspring, however, are shown in two branches, A and B.

Note how in branch B, every mammary gland carcinoma metastasized into the lungs; even the retroperitoneal and pelvic sarcomas (male 3951) metastasized in the lungs. Note also that with the exception of male 1775 this branch shows 100 per cent of lung tumors, primary and secondary. Note the two generations of metastasis into the diaphragm, female 1722 and male 3098.

On the other hand, in branch A, carcinomas of the same type and of older and larger growth, failed to metastasize into the lungs (females 4328, 5723, and 7544). Note that in female 5723, although the mechanical basis for lung secondaries was present in multiple tumor emboli throughout the lungs, no lung tumor developed. Note that the sarcoma of the mesentery in male 4630 metastasized into the liver, kidney, and retroperitoneal glands, but not into the lungs.

Note the three consecutive generations of leukemia in this strain, in males 899, 1785, and 2360; and the further fact that, although the lungs are one of the principal organs for leukemic infiltration, in males 1785 and 2360 there was no infiltration in the lungs, while in male 899 the lung infiltration was very slight. On the other hand, the marked leukemic infiltration in this family was in the same organs in which the tumors of the parent female occurred, that is, the liver and kidney. The metastatic tumors, also, in this family were in these same organs.

In branch B, however, the 100 per cent lung tumor strain, female 6568 who had pseudoleukemia along with an adenoma of the lung, showed marked infiltration of pseudoleukemia throughout the lungs.

We have here, in these two branches of the same strain, a striking illustration of point II: In branch B all tumors metastasize into the lungs, and pseudoleukemia picks out the lungs predominantly; while in branch A, the tumors of the same type in the same organ and of older and larger growth fail, in every case, to metastasize into the lungs. Even the leukemic infiltration fails to take hold of the lungs in this branch.

Chart 10 shows part of strain 405. In this branch of the family, after the parent generation, although there are five straight generations of mammary gland carcinoma, none of these tumors metastasized into the lungs. Note also the complete absence of primary lung tumors. This branch of the family

PART OF STRAIN 405

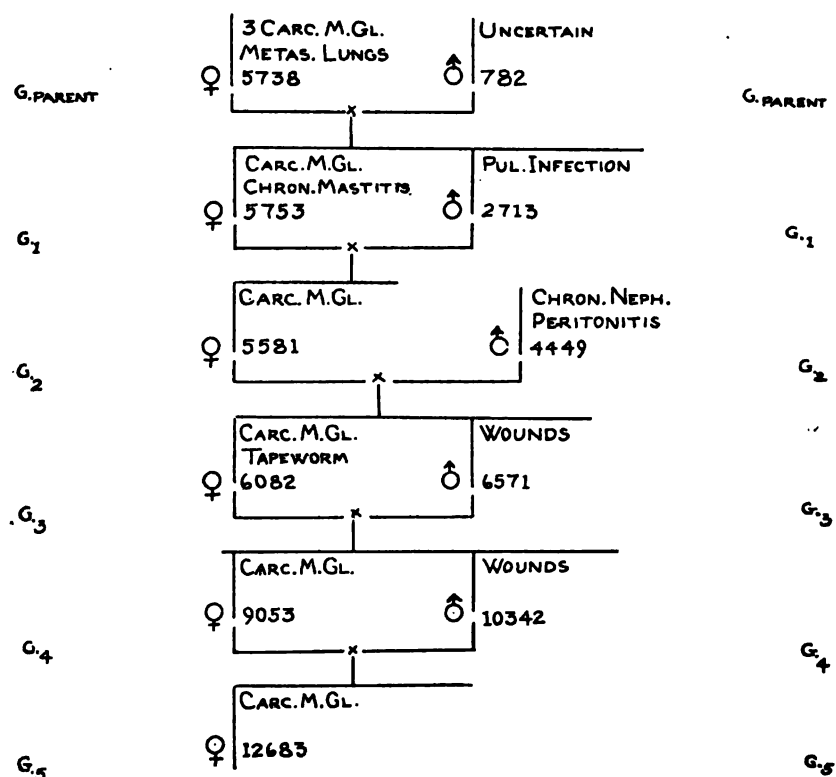


CHART 10

did not inherit lung tumors, and none occurred, either primary or secondary.

Chart 11 shows one branch of strain 508. Here again through five consecutive generations of mammary gland carcinoma, no

lung metastasis occurred. Even where the presence of multiple tumor emboli in the lungs gives certain evidence that cells from the primary tumor were carried to the lungs (in females 7821 and 9260) no lung tumor occurs. The mechanical basis was there; the tumor growth did not follow. This strain also shows no primary lung tumors.

PART OF STRAIN-508

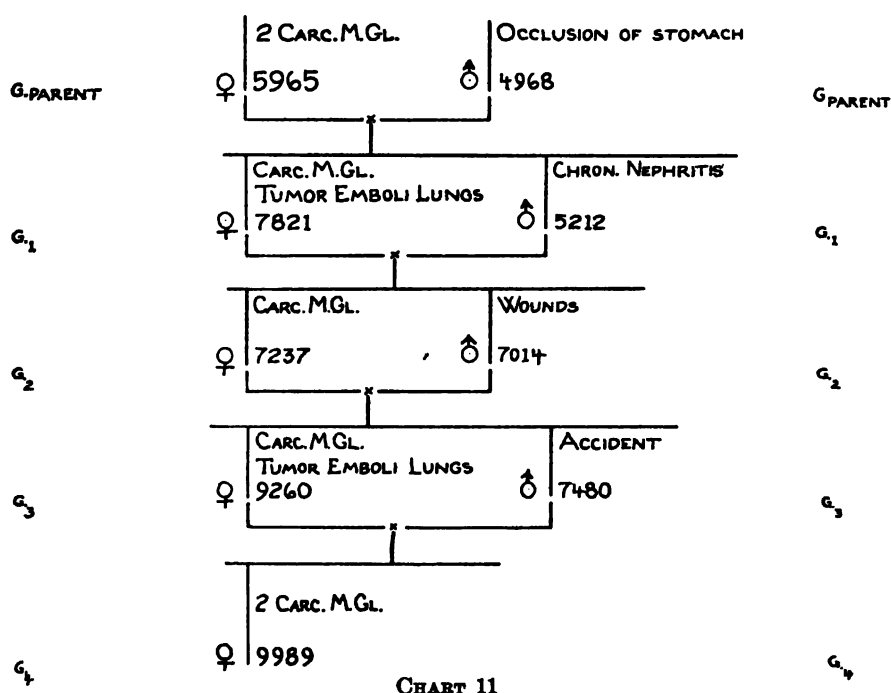


Chart 12 gives a summary of strain 164, branch IV, families A, B, and C. It is shown in summary only in order to get the data within a single chart. These branches of the family showed 48 primary tumors, all but one of them highly malignant, with only one case of lung metastasis, in female 10574, a member of line C. Note the marked absence of lung tumors primary and secondary, although lung tumors are second in frequency of all mouse tumors.

The strain also showed 13 cases of pseudoleukemia and one of leukemia. Here again, it is most interesting to note that the six cases of pseudoleukemia which occurred in line B showed not the slightest infiltration in the lungs. The malignant thymus

STRAIN 164-BR.IV-A-B-C		
PRODUCED THE FOLLOWING CASES OF TUMOR		
WITHOUT METASTASIS		WITH METASTASIS
CARCINOMA M.GL.	34	1 IN LUNG
SARCOMA M.GL.	2	0
OVARIAN ADENOMA	6	0
MALIG. THYMUS LYMPHOMA DID NOT INFILTRATE LUNGS	2	0
LYMPHO-SARCOMA THYMUS DID NOT INFILTRATE LUNGS	1	0
SQ. CELL CARC. BACK	1	0
SP. CELL SARC. BACK	1	0
SQ. CELL CARC. FACE	1	0
SP CELL SARC. FACE	1	0
RETROPERITONEAL SARCOMA	1	0
TOTAL	48	1
PSEUDO LEUKEMIA	14	
NOTE THE ABSENCE OF PRIMARY LUNG TUMOR AND THE SINGLE CASE OF LUNG METASTASIS		

CHART 12

tumors in this line of the family also failed to infiltrate the lungs, although they went extensively into the chest wall and mediastinal glands. The seven cases of pseudoleukemia which fell in line C of this strain, showed a very slight infiltration of the lung. This is the same line which showed one case of lung metastasis.

We have then, in this strain, lines A and B, a marked illustration of the failure of the lungs to yield at all to tumor, primary or secondary. In other words, the lungs of this family do not show neoplastic proliferation, nor do they accept neoplastic secondaries or leukemic growth in spite of the certain and marked presence of the ordinary provocation for such proliferation. In line C of the strain, a slight tendency of the lung to proliferation is present.

This strain was derived by the hybridization of an absolutely nontumorous strain of house mice, in my hands many years, strain 358, with albino strain 146, which carried many tumors, and furnished some 100 per cent tumor families. This strain gives another marked illustration of the segregating out of these things; the tendency to neoplastic lung proliferation, either primary or secondary, failed to be transmitted at all to lines A and B of branch IV of the strain, and only slightly to line C. Note the striking contrast between this strain with no lung tumors, and strain 522 (chart 15) showing 100 per cent lung tumors (p. 167).

These four strains represented in charts 9 to 12 inclusive (which are perfectly typical) furnish both positive and negative evidence of the segregating out of different types and locations of tumors, and their transmission as such, whether as primary or as secondary neoplasms.

They furnish therefore evidence that *in certain strains there is a tendency for tumors to metastasize into certain organs, whereas in other strains, tumors of the same type in the same organ, even when they are of older and of larger growth, fail to metastasize into those organs.* Moreover in these strains, leukemia and pseudoleukemia tend to infiltrate the same organs which show primary and secondary neoplasms, and fail to take hold of the same organs which do not yield to primary or secondary tumors.

In this connection, it is interesting to analyze the results of various experiments to test the effect upon metastasis formation of making exploratory incisions into tumors, of massaging tumors, etc. Let me indicate what would be a complete biological control in such experiments.

TWO ILLUSTRATIONS OF COMPLETE BIOLOGICAL CONTROL.

I

Strain 164 branch C of the Slye stock, during its twelve years of existence has consistently yielded 0.01 per cent of lung tumors primary and secondary, leukemic and pseudoleukemic infiltration of the lung, and infiltration into the lungs from malignant thymus tumors.

If now we use tumorous mice from strain 164 branch C, and excise for diagnosis pieces of their tumors or massage their tumors or otherwise manipulate them, and if we find at autopsy that the group of mice whose tumors have been manipulated show little or no higher percentage than 0.01 per cent of lung tumor metastasis, and that the group which is not manipulated runs true to this 0.01 per cent lung tumor metastasis, we shall have a perfect control, and shall be justified in saying, without qualification, that excising a piece of tumor for diagnosis or massaging the tumor, or otherwise manipulating it, had no effect upon the production of lung tumor metastasis.

II

Strain 65 branch II B has during its twelve years of existence produced 35 per cent of lung tumor metastasis.

If now we repeat these same experiments with this strain, and find at autopsy that the group whose tumors have had a piece excised for diagnosis or which have been in any other way manipulated, show little or no increase in lung tumor metastasis over 35 per cent, and if the unmanipulated group runs true to this percentage of lung tumor metastasis, we shall have a perfect biological control of the experiments, and shall be justified in saying without qualification that the excision of a piece of tumor for diagnosis or any other form of manipulation had no effect upon the production of lung tumor metastasis.

THREE ILLUSTRATIONS OF THESE EXPERIMENTS WITH INADEQUATE
BIOLOGICAL CONTROL

I

Strain 164 branches A and B have during their existence for twelve years, without exception of any sort, yielded 0 per cent of lung tumors primary or secondary even where tumor emboli were present in the lungs, 0 per cent of leukemic or pseudo-leukemic infiltration into the lungs, and 0 per cent of infiltration into the lungs by extension from thymus, diaphragmatic, or other adjacent malignant tumors.

If now we repeat these same experiments with these branches of the strain, we shall find at autopsy 0 per cent of lung tumor metastasis in every group of mice, whether or not we excise a piece of tumor, or massage the tumor, or otherwise manipulate it. That is, a strain whose lungs have been proved not to yield to tumor emboli is not a strain fitted to show the effect of these or any other types of manipulation.

II

Strains 139, 522, 73, branch B, etc., have during their many years of existence without exception furnished 100 per cent of lung tumors, primary and secondary, infiltration into the lungs by leukemia and pseudoleukemia, and extension infiltration into the lungs from malignant thymus and other adjacent tumors.

If now we repeat these same experiments with these or any other 100 per cent lung tumor strains, we shall find at autopsy an exceedingly high percentage of lung tumor metastasis, probably 100 per cent in every group whether excised or not. That is, a strain which for twelve years has shown 100 per cent of lung tumor is not a strain fitted to show the effect upon lung metastasis of this or any other type of manipulation.

III

Groups of mice even when purchased from one dealer are mixed lots, secured from many divergent sources, and wholly unanalyzed

as to tumor potentiality. They may include some individuals from 0 per cent lung tumor strains, some from 100 per cent, some from 35 per cent, etc. If we take such groups of mice and repeat these same experiments, we shall have *no biological control*, and we shall not be justified in saying whether the lung tumor metastases found at autopsy were or were not caused by the excision of a piece of tumor for diagnosis, or by massage, or whatever form of artificial manipulation may have been used.

It is greatly to be deplored that the vast majority of experimental studies in animal pathology and bacteriology have not been and are not as yet being conducted with animals whose hereditary potentialities have been studied. It is being taken for granted that pathological conditions found in the animal after experimental procedure, are the result of such experimental procedure. No allowance is being made for heredity.

Until stocks of animals to be used in such experiments, have been thoroughly tested out as to their inherited potentialities, such experiments are lacking in adequate control, as the factor of heredity is not being considered, although it is tremendously potent. Heredity alone would be sufficient to give the exact results attained in countless experiments which have been conducted without any effort to study or control the heredity factor. Moreover, most of these studies in metastasis production by the artificial manipulation of the primary tumor have been conducted with grafted tumors. As has frequently been stated in the series of cancer studies from this laboratory, the biological difference between spontaneous and grafted tumors is so fundamental and so complete, that the behavior of grafted tumors has practically no bearing upon the behavior of spontaneous tumors.

POINT III

Individuals with secondary tumors in any given organ seem to be as potent as individuals with primary tumors in the same organ to transmit by heredity primary tumors in that organ.

Note chart 13, showing part of strain 304 with ancestry. Here two successive generations of females (529 and 467) with second-

ary lung tumors, have originated a strain which, in line C, is producing a 44.4 per cent primary lung tumor strain. The combination of both lines B and C shows 33.3 per cent of primary lung tumors. Note the 100 per cent pseudoleukemia family being extracted in branch B, in all of which the lungs were infiltrated extensively.

Again, in chart 14, strain 338 branch VI, a 100 per cent lung tumor strain is being extracted from the original mating of female

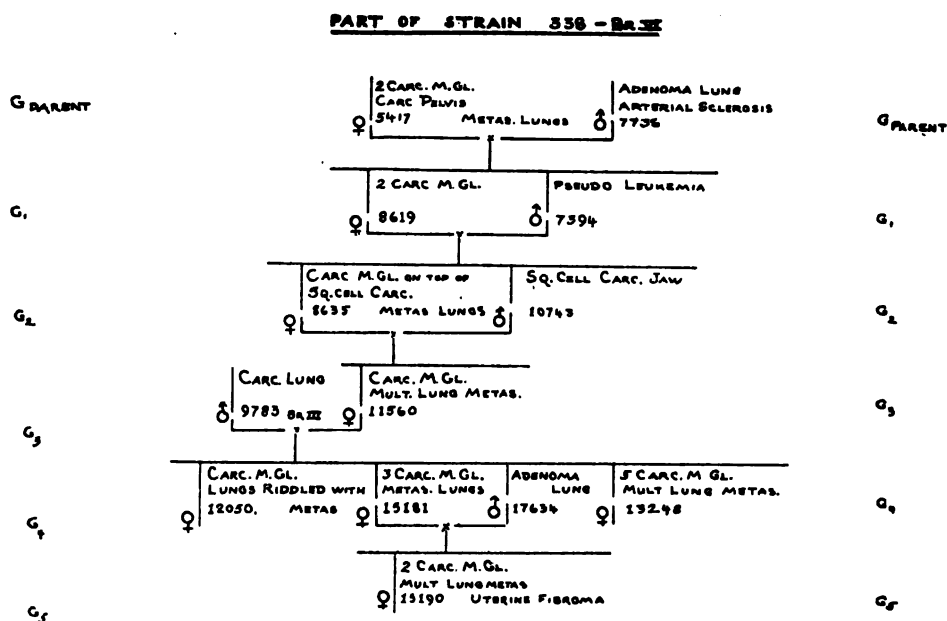


CHART 14

5417, with a *secondary* lung tumor, with male 7736, with a primary lung tumor; that is, the metastatic lung tumor is behaving like a primary lung tumor in helping to produce a 100 per cent lung tumor strain, primary and secondary.

Refer again to chart 1 (page 142), showing part of strain 215, and note how female 630 (parent of branch B) with a metastatic carcinoma of the liver, produced three straight generations of primary liver adenoma (not yet malignant) females 8871, 11237,

and 12207, as well as a primary malignant adenoma of the liver in male 2327. Note also how her secondary lung tumor was followed by a primary lung tumor in female 823 (G_3).

Refer again to chart 2 (page 145), showing strains 48 and 292, and note how the secondary sarcoma of the kidney in parent female 3, resulted in primary sarcomas of the kidneys in females 26, 348, and 396. Refer again also to chart 3 (page 147), strain 65, and note how secondary lung tumor in the parent female 5738, introduced primary lung tumors in the strain, in females 1253, 8749, 11512, and 13386, and in male 11832. Note that the thymus sarcoma in female 9888 was markedly infiltrating the lungs, while in strain 164 Br. IV, lines A and B, which did not produce lung tumors of any sort, either primary or secondary and in which leukemia and pseudoleukemia failed to infiltrate the lungs, the thymus tumors also failed to infiltrate the lungs.

Note also how in chart 15, strain 522, female 5636, with a secondary lung tumor (G_2), mated with male 8102 with a primary lung tumor, produced a 100 per cent primary lung tumor strain (center of the chart). The pseudoleukemia in this 100 per cent lung tumor strain, viz., female 14033, showed marked infiltration in the lungs.

From these charts, viz., 13 to 15 inclusive, and also from charts 1, 2, and 3 (which are perfectly typical) it is evident *that individuals with secondary tumors in any given organ seem to be as potent as individuals with primary tumors in that organ, to transmit by heredity, primary tumors in that organ.*

DISCUSSION

We have here, in the charts presented in this report, biological evidence of the nature of cancer heredity. It is well to bear in mind that until the facts of pathology have been based upon the more fundamental biological facts, it will never be possible to get a complete explanation of the pathological behavior of tissues.

Let me repeat at this point, what I already have stated frequently in previous publications: the materials used for this study are individuals analyzed as to their hereditary poten-

PART OF STRAIN 522 WITH PARTIAL ANCESTRY

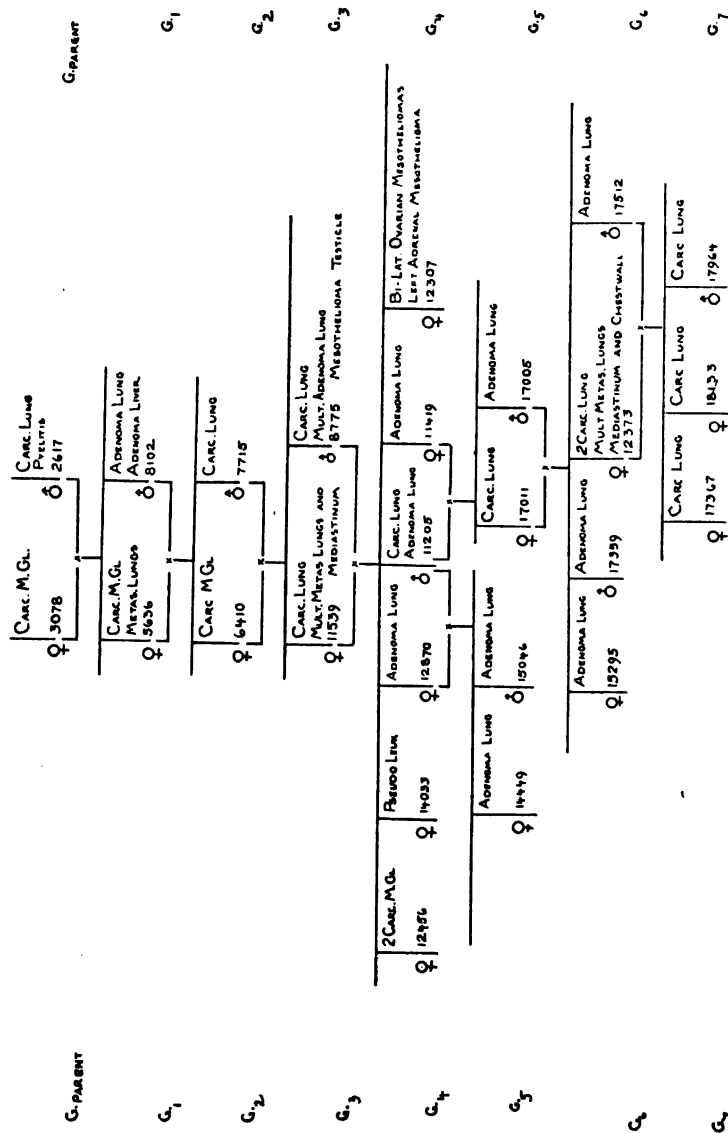


CHART 15

tialities by every possible test. The tumors are all spontaneous, arising without interference of any sort except that of selective breeding. By the process of selective breeding alone, using analyzed individuals as material, it is possible to extract strains in which the lungs do not react with either primary or secondary neoplastic proliferation or leukemic infiltration; or strains in which the liver does not so react, or the kidneys, or the mammary gland tissue, etc.

Etiological meaning. The fact that both primary and secondary tumors of a given organ or organs tend to occur in the same strains, and fail to occur at all in other tumor strains, indicates beyond question that *heredity is a strong factor in determining not only where the primary tumors shall occur, but also where the secondary tumors shall occur.*

The consequent fact, that it is possible to extract strains where only certain organs shall furnish both the primary and the secondary neoplasms, and shall yield to leukemia and pseudoleukemia; and to extract other strains in which these organs never furnish either primary or secondary neoplasms nor yield to leukemic or pseudoleukemic invasion, and that secondary tumors are just as potent as primary tumors in the transmission of primary tumors in any given organ, shows beyond a doubt that the thing which is transmitted in the heredity of cancer is the tendency of a given organ or organs to yield to cancer. This inherited tendency of an organ or organs to yield to cancer is manifested, whether the lesion is primary in that organ, or whether cells from the primary growth lodge in that organ and form a secondary lesion.

The negative evidence on this point is just as conclusive, that is, that in strains from which tumors of a certain organ or organs have been eliminated by heredity, the cells from the primary neoplasm fail to take hold, even when they lodge in such organ, and do not form a secondary growth; and even when a tumor completely surrounds an organ, it fails to penetrate it by extension.

Now what do these things mean? They mean that the tendency to carcinoma segregates out and is transmitted as such;

that the tendency to sarcoma or adenoma, etc., segregates out and is transmitted as such; that a strong tendency to the location of one or more of these types of tumor in a specific organ, such as the liver, the lung, the kidneys, the mammary glands, etc., is transmitted, owing to the segregating out of a peculiar type of tissue in these organs, which will respond in a neoplastic manner to lesions of any kind which furnish a chronic irritation of not too destructive a type. That is, *the tissues of these organs are of the same nature as the ancestral organs from which they are derived. There is a specificity of tissue type from liver to liver, or from kidney to kidney, etc., which will make those organs react in a given way to a given type of irritation.*

Now with each of these things being transmitted as such, it is possible for a parent female with a sarcoma-carcinoma of the mammary gland, a malignant adenoma of the liver, and a secondary sarcoma of the kidney (like female 3) to transmit to the strains derived from her, a tendency to:

1. Carcinoma of the mammary gland.
2. Sarcoma of the mammary gland.
3. Adenoma of the mammary gland.
4. Carcinoma of the liver.
5. Sarcoma of the liver.
6. Adenoma of the liver.
7. Carcinoma of the kidney.
8. Sarcoma of the kidney.
9. Adenoma of the kidney, or any combination of these nine.

If she is mated with a male, either himself having a lung and a mediastinal tumor, or being heterozygous to these locations of tumor (as in the case of male 360), the resulting strains will show:

1. Carcinoma of the mammary gland.
2. Sarcoma of the mammary gland.
3. Adenoma of the mammary gland.
4. Carcinoma of the liver.
5. Sarcoma of the liver.
6. Adenoma of the liver.
7. Carcinoma of the kidney.
8. Sarcoma of the kidney.

9. Adenoma of the kidney.
10. Carcinoma of the lung.
11. Sarcoma of the lung.
12. Adenoma of the lung.
13. Carcinoma of the mediastinum.
14. Sarcoma of the mediastinum.
15. Adenoma of the mediastinum, or any combination of these tumors.

The neoplastic growth may be primary in any one or more of these organs, according to where there is occasion for the primary lesion to occur, and there may be secondary growths in any one or more of these organs, according to where the secondary lesions occur.

Now note that this is exactly what we get from the matings of female 3 (with a sarcoma-carcinoma of the mammary gland, a malignant adenoma of the liver, and sarcoma metastasis in the kidney) with male 360, proved heterozygous to lung and mediastinal tumors. Note especially charts 1 and 2 where the strains were derived from the same original mating of female 3 with male 360; and charts 4, 5, 6, 7, 8, and 9, showing ancestry and strains derived from the mating of female 3 with male 30.

It should not be a matter for surprise that there is an inheritance of a specific type of liver tissue, or kidney tissue, etc., which will tend to respond in a given way to a given irritation or a given lesion. Similar facts of inheritance, such as a tendency to grow tall, with the proper degree of nourishment, etc., are easily accepted. That also is the inheritance of tissues which tend to respond in a given way to a given stimulus. There is here also a certain specificity, as, for example, the tendency to longness in the trunk and shortness of the limbs; or of shortness of the trunk and longness in the limbs, so that a given figure tends to predominate in a given family, just as given types and locations of neoplasms tend to predominate in a given family. Again, it is an accepted expectation that there should be a given nose shape, or shape of lips, or contour of head, inherited within a family; i.e., a specificity of nose tissue, etc., which will insure its growing to a given shape, etc.

This specificity of organ tissue, which shall insure its reacting in a given neoplastic way, to a given chronic irritation, is exactly what we find to be the nature of cancer heredity; and it is obvious that it will make no difference in the nature of the reaction of such specific organ tissue, whether the lesion is primary or secondary.

Heredity, therefore, of a specific type of organ tissue, is here shown to be the fundamental influence in determining the incidence and location of metastatic neoplasms, as well as those of primary neoplasms.

In regard to the relative infrequency of secondary tumors in this stock, the biological evidence is as follows: Many of these strains have been made by hybridizing a tumorous individual with a proved non-tumorous individual. This, of course, is done to test for the Mendelian behavior of cancer in heredity. Now, with the tendency to carcinoma, sarcoma, etc., segregating out, and a specific type of organ tissue segregating out and being transmitted as such, it is evident that any given mouse may inherit a tendency to only one type of tumor, and in only one organ. Other organs, therefore, in such a mouse, refuse to yield to cancer, even where emboli are present in such organs. Many such cases have been pointed out as shown in the charts in this report.

Individuals into whose ancestry (as is the case in much human heredity) an indiscriminate amount of cancer had been bred would show a correspondingly higher percentage of metastasis.

SUMMARY

These studies in the metastasis behavior of spontaneous tumors demonstrate the following facts:

1. In any given strain, the metastatic tumors (where there are any) tend to occur in exactly the same organs in which the primary tumors of that strain occur.
2. In certain strains, there is a tendency for tumors to metastasize in certain organs; whereas in other strains, tumors of the same type in the same organ, even where they are of older and of larger growth, fail to metastasize into those organs.

3. Leukemia and pseudoleukemia, occurring in tumor strains, pick out predominantly the same organs for infiltration which show the primary and secondary tumors of that strain.

4. Tumors do not even invade by extension the organs from which primary and secondary neoplasms have been eliminated by heredity.

5. Individuals with secondary tumors in any given organ, seem to be as potent as individuals with primary tumors in the same organ, to transmit by heredity, primary tumors in that organ.

Therefore:

6. Heredity is a strong factor in determining not only where the primary tumors of a strain shall occur, but also where the secondary tumors shall occur.

7. Heredity is a strong factor in determining what organs of a strain shall yield to the invasion of leukemia and pseudoleukemia.

8. The thing which is transmitted in the heredity of cancer is the tendency of an organ or organs to yield to cancer. This tendency is manifested whether the lesion is primary in that organ, or whether cells from the primary growth lodge in that organ and form a secondary lesion.

9. The tendency to sarcoma, carcinoma, adenoma, etc., segregates out and is transmitted as such.

10. A strong tendency to the location of one or more types of tumor in a specific organ or organs, such as the liver, kidney, pancreas, mammary gland, etc., is transmitted, owing to the segregating out of a peculiar type of tissue in these organs, which will respond in a neoplastic or leukemic manner to lesions of any kind which furnish a chronic irritation of not too destructive a type.

11. That is, there is a specificity of tissue type, from organ to organ in a strain, which will make these organs react in a given way to a given type of irritation.

12. It is, therefore, possible for ancestry to transmit to its posterity every possible combination of the neoplastic or leukemic tendencies which they carry either actually or potentially.

13. This specificity of tissue type in organs, which will insure its reacting in a given neoplastic manner or accepting leukemic deposits, in response to a given chronic irritation, is what we find to be the nature of cancer heredity, and it is obvious that it will make no difference in the nature of the response of such specific organ tissue, whether the lesion is primary or secondary in that organ or organs.

Heredity, therefore, of a specific type of organ tissue, is here shown to be the fundamental influence in determining the incidence and location of secondary tumors and of leukemia and pseudoleukemia, just as it is in determining, the incidence and location of primary neoplasms.

It is also pointed out that any apparent testimony of the frequent occurrence of secondary tumors in man in tissues where primary tumors rarely occur, would be of no help here, as no human strain has ever been even partially analyzed, and no right conclusions regarding heredity can be drawn except from analyzed strains.

It is also suggested, that until stocks of animals to be used in pathological and bacteriological experiments, have been thoroughly tested out as to their inherited potentialities, such experiments will be lacking in any adequate control, since heredity is not considered although it is tremendously potent.

REFERENCES

- (1) EWING: Neoplastic Diseases, Philadelphia, 1919, p. 86.
- (2) SLYE, HOLMES, AND WELLS: J. Cancer Res., 1919, iv, 207.
- (3) SLYE, HOLMES, AND WELLS: J. Cancer Res., 1917, ii, 401.
- (4) SLYE, HOLMES, AND WELLS: J. Cancer Res., 1921, vi, 57.
- (5) SLYE, HOLMES, AND WELLS: J. Cancer Res., 1917, ii, 14.
- (6) LOEB, L: Univ. Penn. Bull., 1906, xix, 113.
- (7) LEWIN: Ztschr. Krebsforsch., 1908, vi, 273.
- (8) BASHFORD, et. al: Third Sci. Report, Imperial Cancer Research Fund, 1908, 322.
- (9) WOGLOM: J. Cancer Res., 1918, iii, 47.
- (10) LECOUNT: Reference in J. Cancer Res., 1917, ii, 6.
- (11) EWING: Neoplastic Diseases, Philadelphia, 1919, p. 143.

PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH

FOURTEENTH ANNUAL MEETING

Held in Cleveland, Ohio, March 24, 1921

1. REPORT OF THE COUNCIL

The meeting of the Council was held at the Hotel Statler in Cleveland, Ohio, on the evening of Wednesday, March 23, 1921.

The following members were present: Dr. Robert H. Greenough, president; Dr. Francis C. Wood, and Dr. William H. Woglom. Absent, Dr. James B. Murphy, Dr. E. R. LeCount, Dr. Willy Meyer, and Dr. James Ewing. As four members of the Council are required for a quorum, business was transacted temporarily with the idea of obtaining subsequently the vote of another councillor. Dr. Murphy's vote sustaining the action of the Council was subsequently received by mail.

The treasurer's report was read and accepted.

The following officers were elected to serve for the ensuing year: Dr. James B. Murphy, president; Dr. Willy Meyer, vice-president; Dr. William H. Woglom, secretary and treasurer (re-elected).

Dr. H. Gideon Wells was elected Councillor to succeed Dr. E. R. LeCount, whose term of office expired.

The present Council, therefore, with the years of retirement, is as follows:

Dr. F. C. Wood, 1922	Dr. Robert B. Greenough, 1925
Dr. James B. Murphy, 1923	Dr. Willy Meyer, 1926
Dr. William H. Woglom, 1924	Dr. James Ewing, 1927
Dr. H. Gideon Wells, 1928	

The present Editorial Board was continued in office. It is composed of

<i>Editor</i> , Dr. Woglom	
<i>Associate Editor</i> , Dr. Prime	
Dr. Bloodgood	Dr. Loeb
Dr. Wells	Dr. Ewing
Dr. Tyzzer	

The following resignations were accepted:

Dr. C. L. Alsberg.....	Associate
Dr. D. B. Phemister.....	Associate
Dr. Robert G. Leconte.....	Associate
Dr. F. P. Gay.....	Active

There has been one death during the year—Dr. H. H. Janeway, an active member, who died on February 1, 1921.

The following gentlemen were elected to membership:

Active

Dr. Herbert U. Williams, Univ. of Buffalo
Dr. David Marine, Montefiore Home and Hospital
Dr. William Ophuls, San Francisco, Calif.
Dr. H. T. Karsner, Cleveland, Ohio
Dr. Wm. Bayard Long, New York
Dr. Leo Buerger, New York
Dr. James H. Wright, Mass. General Hospital
Dr. Carl V. Weller, Ann Arbor, Mich.
Dr. Georgine Luden, Mayo Clinic
Dr. Henry Albert, Univ. of Iowa
Dr. Eugene L. Opie, Washington Univ. Sch. of Med.

Associate

Dr. John G. Clark, Philadelphia, Penn.
Dr. Stuart Graves, Louisville, Ky.
Dr. Harris P. Mosher, Marblehead, Mass.
Dr. Kenneth T. Taylor, New York
Dr. G. R. Minot, Boston
Dr. Seth Milliken, New York
Dr. John L. Yates, Milwaukee, Wis.
Dr. Charles Norris, Chief Med. Examiner, New York
Dr. D. Crosby Greene, Boston
Dr. F. S. Mandlebaum, New York
Dr. Hugh H. Young, Johns Hopkins Hospital
Dr. Henry A. Christian, Peter Bent Brigham Hospital
Dr. Otto V. Huffman, New York
Dr. L. Duncan Bulkley, New York
Dr. Joseph A. Blake, New York
Dr. Ward J. MacNeal, Forest Hills, N. Y.
Dr. Channing C. Simmons, Boston, Mass.
Dr. Otto Krehbiel, New York
Dr. Julius Rosenstirn, San Francisco, Calif.

The application of Mr. Donald C. A. Butts, who has not yet published any articles on cancer, was laid on the table, and the application of Dr. Rex Duncan was postponed.

SCIENTIFIC SESSION

A letter from Dr. H. J. Conn, Chairman of the Committee on Bacteriological Technique, of the Society of American Bacteriologists, calling on biologists to coöperate with that Society in helping to secure a reliable domestic source of stains was brought to the attention of the Association of Cancer Research at its scientific meeting on March 24, by the President, Dr. Robert B. Greenough.

It was moved by Dr. Wood and seconded by Dr. Gaylord that the Association encourage the manufacture of American dyes. The motion was carried.

2. REPORT ON THE JOURNAL OF CANCER RESEARCH

Dr. F. C. Wood (New York):

SUMMARY

It has seemed to the Council a pity to allow the JOURNAL OF CANCER RESEARCH to lapse, inasmuch as the volumes so far printed contain most important papers on cancer research, and when a journal ceases publication the libraries put the bound volumes on the back shelves and no one ever sees them again, unless specially inquired for. The Association has resigned all responsibility for the JOURNAL OF CANCER RESEARCH and the Crocker Fund, Columbia University, has assumed it. There will be no change in form or publishers at the present time. The Crocker Fund will have to meet an annual deficit in the cost of publication and I hope the members of the Association will do all they can to increase our subscription list, because unless this is done the Crocker Fund will have to continue the present practice of the Editorial Board and charge pretty heavily for cuts and reprints.

If we could get an additional three or four hundred subscribers it would greatly reduce the cost of publication to the individual writers.

DISCUSSION

Dr. Robert B. Greenough (Boston): The Council felt that the Crocker Fund is coming to the rescue of the Association in the matter of the JOURNAL OF CANCER RESEARCH and that there is a distinct obligation upon this Association that the JOURNAL should not be abandoned; for much of the work in the past six or seven years in this country has been published in this JOURNAL. If abandoned, the material in the earlier editions is lost and not readily accessible to new investigators. It is thus an obligation to the contributors that the JOURNAL should be continued, and apparently only by means of assistance from the Crocker Fund can this be done. For these reasons the offer of the Crocker Fund was accepted.

3. FURTHER STUDIES IN RADIATION DOSAGE

Dr. F. C. Wood and Dr. Frederick Prime (New York):

SUMMARY

Dr. Wood showed a series of lantern slides illustrating the fact that, contrary to statements emanating from Germany, there is no destructive carcinoma or sarcoma dose; that is, no fixed amount of x -ray can be assumed to destroy the cells of any one tumor, for apparently the dosage differs greatly both in man and animals for the same microscopic type of tumor.

As the charts showed, in animal tumors the required dosage is from 2 to 8 erythema doses, and the sarcomata vary in resistance as well

as the carcinomata. One interesting thing is the greatly delayed appearance of tumors at a point where the cells received a sublethal dose. In a mouse tumor which ordinarily appears within a week and gains a fair size in two weeks, the appearance may be delayed for a month or more and the tumor grow very slowly afterward. If a transplantation be made from this tumor into another animal the growth rate will rapidly approximate normal. Inasmuch as two or three months in a mouse's life is equivalent to the same number of years in a human being, the question must be raised whether we are not going to see late recurrences, three or four or five years after the symptomatic cure of malignant tumors in man, when such symptomatic cure is obtained by either radium or *x*-ray.

Most of our experiments have been made by raying the tumor particles *in vitro* and then transplanting them; if the tumor be rayed in the mouse the animal is killed in a few days by the radiation. If the tumor be immediately transplanted it grows in the new host, unless it has been given a lethal dose. This lethal dose is approximately 10 to 20 per cent more than when the cells are exposed *in vitro*.

DISCUSSION

Dr. E. T. Bell (Minneapolis): What is your opinion of the treatment of cancer of the cervix uteri with very high voltage apparatus such as is now in use in Germany?

Dr. F. C. Wood: Replying to Dr. Bell's inquiry, whether rays from new high voltage machines are more effective than those from the older type of apparatus, Dr. Wood said he had not found that there is any increase in the destructive action of rays given off by tubes running at 180,000 volts as compared with those running at 120,000 volts, measuring peak voltages between 12.5 cm. spheres. In all his tests there had been no question of absorption or of scattered radiation, the tumor having been directly exposed to the rays. The advantage of the high voltage machines lay in the greater penetration which can be obtained, but there is no reason to assume that they would be any more effective on superficial growths for equal *x*-ray dosage. Reports received recently from German clinics seem to indicate that some of the published results are greatly exaggerated.

In reply to an inquiry from Dr. Greenough regarding stimulation effects, Dr. Wood said that he had not noticed any increase in growth rate of the tumors, unless very small doses were given; and that the tumors which had been greatly slowed at their first transplantation by large doses, grow at their regular rate in the second transplantation.

In response to an inquiry as to the exact mechanism of the effect of *x*-ray on the cell, and whether the connective tissue does not play an important part in the destruction of the tumor cell, Dr. Wood replied

that he had not the slightest idea of the nature of the changes causing destruction of the cells by x-ray or radium. All we know is that the division mechanism is interfered with, and that cells in a resting phase are much more resistant to radiation than those in mitosis. Experience with animals proves that it takes very large doses to kill all the cells of a tumor; those in the center of the growth are killed partially by direct action and partially by the thrombosis which occurs early in the smaller capillaries. The periphery of the tumor, however, is, as a rule, better nourished and there the cells require maximum dosage. An absolute cure requires the killing of every cell during a single exposure, for if divided doses be given, some cells may recover from the radiation effects. Dr. Wood had not observed that connective tissue is capable of destroying cancer cells. It is perfectly possible that such cells might be inclosed in dense scar tissue and remain quiescent for a considerable period, but there is a possibility that they might again begin to grow years after their inclusion. This had been frequently observed in human tumors in the late recurrences in operation scars. Dr. Wood had seen some such recurrences five, six, seven, or eight years after operation.

In response to an inquiry as to the voltage used, Dr. Wood said that this was 120,000 volts, peak, measured between 12.5 cm. spheres. The tumor fragments used in vitro were about 1 to 2 mm. in diameter. If whole tumors be used, or if the tumor be exposed in the mouse, the superficial layers nearest the tube will be killed, while the deeper layers are not. At this voltage, 10 cm. of tissue absorbed 50 per cent of x-ray incident on the surface; this 50 per cent includes not only directly transmitted rays but also scattered ones. His own experiments had all been devised to avoid the complications brought into the problem by scattering and absorption, the effect of which can be easily determined by an ionization chamber; this varies in human cases with the individual, because of the varying depth of the tumors. Dr. Wood's dosage was obtained in a way which made it independent of such effects.

Dr. William Duane (Boston): The difference between the dosage measured by Dr. Wood and that reported from Germany, may be due to scattering. According to recent reports, at a distance of 10 cm. below the skin scattering may increase the dosage $2\frac{1}{2}$ times. If these pieces of tumor be surrounded by other bits of tissue, they may perhaps receive $2\frac{1}{2}$ times as much radiation as they do under the experimental conditions described by Dr. Wood.

4. HIGH FREQUENCY X-RAY SPECTRA

Dr. William Duane:

DISCUSSION

Dr. Wood: We all know, of course, that these experiments of Dr. Duane and the results which he has shown us, form the foundation of

all our *x*-ray work. They underlie all the measurements of the amount of the *x*-ray which reaches the body; and when his results on the physical side meet mine on the biological side, we shall know the whole story. He is investigating the methods of measurement of the quality and quantity of *x*-ray which comes through our filters, while I am trying to determine how much of what he thus has measured is necessary to kill a cell. In a few years we shall know the effect of long rays or short. The one question which the practitioner has to decide is, whether the human body will stand all the radiation necessary to kill all the cancer cells. If it will not stand this amount, we cannot cure cancer with radiation; if it will, we can. I recently gave a patient something like 45 erythema doses for a melanoma of the foot; the blood changes were nominal; the only effect was that the patient was somewhat nauseated. The foot was of necessity removed within twenty-four hours to avoid absorption effects from the dead tissue. This test shows that such a dose can be given on a part of the body where the rays do not penetrate important organs; but when it comes to putting large doses into the abdominal cavity it is a different question. The question of distance is also important. If we could only get a tube such that we could place the patient at a distance of a meter, conditions would be better than at present, where we work with a distance of 30 or 40 centimeters. The next step is improvement in the wattage capacity of the tube. Somebody must design a tube which will stand longer quantities of current than we can now employ. The amount of energy is very small, and exposure, therefore, amounts to six or eight hours, which is too much for a patient to stand. If we cannot contrive to shorten the exposure by use of a very powerful tube, we shall not get much farther ahead than we are at the present time.

Dr. Duane: The *x*-ray energy increases as the square of the voltage. When the voltage is doubled, four times as much radiation is obtained, thus decreasing the length of time required for exposure.

5. THE OCCURRENCE OF SQUAMOUS-CELL CARCINOMA IN MICE

Miss Maud Slye, Miss Harriet F. Holmes, and Dr. H. Gideon Wells (Chicago): Presented by Dr. H. Gideon Wells:

SUMMARY

Review of the literature on the comparative pathology of carcinoma shows that, in general, squamous carcinomas are not so common in other animals as in man, with the possible exception of the dog, in which carcinoma of the skin is not infrequent. This infrequency in animals is probably more real than statistics indicate, since the skin carcinoma will usually be recognized, whereas the great majority of internal growths will escape attention. In horses, carcinoma of squamous character is seen occasionally about the external genitalia of both sexes.

Fowls and other birds not infrequently show carcinoma of the skin, and such growths have also been observed in fish and amphibia. A few tumors of this group have been described in rats, and squamous-cell tumors of the mouth and skin have been observed in mice as well as a few cases of squamous-cell carcinoma arising from the cardiac end of the stomach. Most of the squamous-cell carcinomas described in mice have occurred in the mammary gland, apparently arising either from the ampullae of the lacteal ducts or by metaplasia of a columnar cell carcinoma.

In 28,000 consecutive autopsies performed on mice of the Slye stock, which had been permitted to live as long as possible without any experimental manipulations whatever, we have observed the following instances of primary neoplasms of squamous or stratified epithelial structure.

Primary squamous-cell carcinoma of the skin and mouth.....	71
Primary basal-cell carcinoma of the skin.....	15
Primary keratinizing cell carcinoma of the mammary gland.....	56
Primary squamous-cell carcinoma of the stomach.....	4
Primary keratinizing cell carcinoma of the lung.....	1
Primary squamous-cell carcinoma of the rectum.....	2
Primary squamous-cell carcinoma of the vagina.....	1
Primary stratified carcinoma of meibomian gland.....	2
Primary sebaceous gland adenocarcinoma.....	1

153

The squamous-cell carcinomas of mice are distinguished especially by the infrequency of metastasis and they generally show a relatively slight tendency to infiltrate, although we have observed even infiltration of the skull and spinal column. This probably depends on the fact that secondary infections usually kill the mice at an early stage of the disease. All the basal-cell carcinomas and the great majority of squamous carcinomas arose about the head, neck, and mouth, these being the sites of the greatest amount of irritation. Frequently they have arisen in healed wounds.

(A full report of this material will be published in a forthcoming number of the JOURNAL OF CANCER RESEARCH.)

DISCUSSION

Dr. Wells: In reply to a question regarding the life span of the mouse, Dr. Wells replied that it depends upon the stock. Miss Slye has a strain of Japanese waltzing mice which become senile before they are a year old. The chief cause of death in old mice is chronic nephritis.

Miss Slye: The average published life of the mouse is 1½ years, but many mice in my laboratory live to be six years old or more; the average age, however, is from three to four years. Every effort is made to keep them alive as long as possible.

Dr. Wells: I may state that these squamous-cell carcinomas in mice arise distinctly at a later age on the average than the other carcinomas. This is true in human squamous-cell carcinomas, too.

Dr. William H. Woglom (New York): In a paper published in the JOURNAL OF CANCER RESEARCH I have discussed the frequency of keratin in mammary carcinoma of the mouse, and have estimated it at about 23 per cent.

6. THE INFLUENCE OF HEREDITY IN DETERMINING TUMOR METASTASIS:
STUDIES IN THE INCIDENCE AND INHERITABILITY OF SPONTANEOUS
TUMORS IN MICE. FIFTEENTH REPORT

Miss Maud Slye:

SUMMARY

Metastases in this stock are somewhat rare. In 29,000 autopsies, furnishing something over 4000 primary spontaneous tumors, about 19 per cent of the growths metastasized. The general testimony of those who have discussed metastasis at all, seems to be that the occurrence and location of metastasis is partly a mechanical matter, and partly a striking specificity of localization of secondary growths. This specificity they make no attempt to explain.

Ewing, speaking of metastasis in human tumors, states that in highly vascular tissues like the lip, stomach, and testicle, very small carcinomas may yield distant metastasis. But these very organs are among those whose tumors rarely metastasize in this stock, and then only locally. For example, of our carcinomas of the lip none has metastasized; of growths in the testis, one metastasized locally; while among carcinomas of the stomach three metastasized into the regional lymph-nodes only. And this in spite of the close resemblance between these neoplasms and human tumors in similar organs.

In this stock mammary gland tumors rarely metastasize into the regional lymph-nodes, in contrast to those of the human breast. Indeed, in many cases in this stock, carcinoma and sarcoma of the mammary gland can be seen growing up to the lymph-node but not invading it. On the other hand, pulmonary metastases from mammary gland carcinoma and sarcoma are common in this stock.

These divergences in the metastatic behavior of tumors of similar type and in similar organs, require some explanation other than a mere mechanical tendency of certain types of tumor to metastasize in certain locations.

Metastasis in this stock has been under observation for ten years and the data here given are based on over 29,000 autopsies.

Briefly stated, these results show conclusively: (1) In any given strain the metastatic tumors tend to occur most frequently in exactly the same organs in which the primary tumors of that strain occur. For example, if a strain is high in primary tumors of the liver, many

secondary tumors will be found in that organ. Again, if a strain yields large numbers of primary lung tumors, metastasis will tend to occur in the lungs from primary tumors in almost any location. A strain high in primary kidney tumors is high also in secondary kidney tumors, etc.

(2) In certain strains there is a tendency for tumors to metastasize into certain organs; whereas in other strains, tumors of the same type in the same organ, even where they are older and of larger growth, fail to metastasize into these organs. For example, in certain strains practically every carcinoma of the mammary gland metastasizes into the lungs; I have many strains showing 100 per cent such behavior. On the other hand, in other cancer strains exactly this same type of mammary gland carcinoma, even where older and of larger growth, never metastasizes into the lungs; and I have many strains showing 100 per cent of this type of behavior. Indeed, even where tumor emboli reach the lungs in these non-pulmonary strains, the emboli fail to take hold and no tumor is formed. This is definite proof that the lung here fails to yield, even when tumor cells are present.

Again, strains which never yield primary lung tumors never yield secondary lung tumor. Strain 164 is a striking example of this, one family yielding 36 carcinomas and sarcomas of the mammary gland without one case of lung metastasis. In this family also there has never been one case of primary lung tumor.

(3) Individuals with secondary tumors in any given organ seem to be as potent as individuals with primary tumors of that organ to transmit (by heredity) primary tumors in that same organ. For example, female 3 with a primary carcinoma-sarcoma of the mammary gland, and secondary sarcoma in the kidney, transmitted primary kidney tumors to the strains derived from her, one family showing as high as 37 per cent of primary kidney tumor.

Furthermore, individuals with secondary lung tumors seem to be just as potent as individuals with primary lung tumors to transmit (by heredity) primary tumors of the lung. Thus, many strains derived from a female with a primary mammary carcinoma with metastasis in the lungs, mated with a male showing primary lung carcinoma (double lung-cancer parentage) show 100 per cent primary lung carcinoma.

(4) Etiological meaning: The fact that both primary and secondary tumors of a given organ tend to occur in the same strains and fail to appear at all in other strains, indicates that *heredity is a strong factor in determining not only where the primary tumors shall occur but also where the secondary tumors shall occur.*

This fact apparently means that the thing which is transmitted in the heredity of cancer is the tendency of an organ to yield to cancer, whether the lesion is primary in that organ or whether cells from the primary growth lodge in that organ and form a secondary lesion. Moreover, in strains from which tumors of a certain organ have been eliminated by heredity, even where cells from the primary neoplasm lodge in such organ they fail to take hold and do not form a secondary growth. It is, therefore, evident that not only primary neoplasms,

but secondary tumors also, are determined in their occurrence and their location by heredity.

DISCUSSION

Dr. G. H. A. Clowes (Indianapolis): Has anybody ever attempted to determine whether any of these strains are more or less sensitive to absence of vitamins?

Miss Slye: I do not think so.

Dr. Clowes: I should think that it might be a very important factor in proliferation.

Miss Slye: I have been carrying on some dietary experiments in my own laboratory, but they are at too early a stage for me to give any data.

Dr. Wells: Dr. Wood who has investigated the development of metastases in rats subjected to exploratory incision, in order to determine the rate of metastasis after such incision, has found distinct variations among control rats of different strains, in respect to metastases. These experiments, however, were carried out with grafted tumors, whereas Miss Slye's work deals entirely with spontaneous growths.

Dr. Greenough: As I understand it, all the matings were made before it was known that female No. 3 had any tumor. Was her tumor discovered at autopsy?

Miss Slye: Female 3 had offspring both before and after the appearance of her tumor. Her mammary tumor was evident some months before her death, while the growths of liver and kidney were found at autopsy. Many of these matings are made before the tumors appear, for if one waits for the appearance of a tumor one is apt to lose the chance of offspring in a large number of cases. Mice of high cancer ancestry should be mated early. In the majority of my cancer mice I have records of offspring both before and after the appearance of tumors. In the matter of tumor inheritance it makes no difference whether the offspring precede by many months the appearance of the growth or are born after its appearance. The grandchild or great-grandchild may develop a tumor before the grandmother from whom the tumor is inherited. In tumors of the liver and kidney it is difficult to make a clinical diagnosis, though this is sometimes possible. Tumors of the lung are frequently accompanied by a peculiar condition of the eye, from which it is often possible to make a clinical diagnosis.

Dr. Clowes: Is there any relation between age and the rapidity at which tumors tend to develop and metastasize?

Miss Slye: The relation of age and tumor growth has not yet been completely worked out, but in general it is true that tumors grow more slowly in old mice, as they do in old men. Also it may be said in general that the tumors of old mice never grow to any great size, and that old mice seldom are able to support more than one tumor.

7. PROOFS OF THE CONSTITUTIONAL NATURE OF CANCER

Dr. L. Duncan Bulkley (New York):

SUMMARY

- | | |
|----------------------------|--|
| I. Laboratory findings | { Negative { Cancer not parasitic
{ Cancer not contagious
{ No cause for cancer
{ Positive { Cancer cell an altered normal cell
{ Feeding experiments showing control
{ of cancer growth |
| II. Statistical evidence | { Control of death statistics of cancer and tuber-
culosis, especially since 1900
{ Steady increase of cancer deaths under surgery,
x-rays, and radium |
| III. Bio-chemical evidence | { Blood in cancer; early and late metabolic changes
in the system before and after the development
of the local cancerous lesion |
| IV. Clinical | { Opinion of many celebrated surgeons during the
last 100 years, to the present time
{ Spontaneous cures of cancer reported
{ Dozens or hundreds of attested cases of benefit
or cure of cancer by numerous physicians, in
this and other countries |

DISCUSSION

Dr. Bulkley: I hope for comments or objections, because if I am wrong I want to know it.

Miss Slye: It might interest Dr. Bulkley and the Association to know that there is a standard diet maintained in my laboratory, which is identical for tumor strains and non-tumor strains. With autopsies at 29,000 and living inhabitants numbering about 13,000, all fed on exactly the same diet, the tumor strains have yielded over 4000 spontaneous tumors, while the non-tumor strains have never yielded a single tumor. No meat is ever used in our laboratory.

Dr. W. T. Bowie (Boston): The mortality from tuberculosis in the state of Massachusetts is of interest in this connection. A curve showing the progressive decrease in mortality deviates but little from a straight line. Its course does not appear to have been changed by any of the activities of the medical profession. Such deviations from a straight line as do occur seem to be correlated with influxes of foreign people.

Dr. Wells: I am familiar with Dr. Mayo's paper on the subject of the influence of diet on gastric cancer, and it seems to me that Dr. Bulkley has mis-interpreted it. Dr. Mayo was discussing the influence of local conditions in causing carcinoma of the stomach, and not the influence of diet in its constitutional relation.

8. RELATION OF HETEROSEXUAL CHARACTERS TO NEOPLASMS AND DEVELOPMENTAL ERRORS

Dr. Otto V. Huffman (New York): Read by title:

SUMMARY

A preliminary report on the association of heterosexual secondary sex characters with neoplasms, especially carcinomata, in patients observed by the speaker. In noting the occurrence of status lymphaticus in the clinic he observed that about ten per cent of the patients showed some degree of heterosexual secondary sex characters, especially in regard to the pubic hair and that on the chest and on the face, and in regard to the width of the chest and of the hips. He found in some of these patients such evidence of maldevelopment as a patent omphalomesenteric duct and gynecomastia. This led him to take note of the secondary sex characters of patients with neoplasms. A marked degree of heterosexuality in the secondary sex characters is not incompatible with normal sexual life and reproduction. Four married patients who developed carcinoma showed slight degrees of heterosexuality but the marriages were sterile. Another four married patients who developed carcinoma and who had some heterosexual characters did not develop their neoplasms until after their active sexual life, that is, after the age of fifty. The greatest degree of heterosexuality was observed in patients who developed carcinoma early, that is, prior to fifty years of age. This may mean that the degree of heterosexuality has some etiological significance. Between the 100% normal male and the 100% normal female we have a whole series of intergrades. Several observers have stated that these intergrades are evidently on the increase. Ordinarily they are more susceptible than the normal to acute infectious diseases, but sanitation and public health measures may be favoring them to such an extent that they mature and thereby arrive at the cancer age. From the author's observations it would seem that early cancer is relatively more prevalent among them than among the normal, but he is not yet prepared to publish comparative tables that would establish this point conclusively.

9. END-RESULT REPORTS OF CANCER OPERATIONS

Dr. Robert B. Greenough and Dr. Channing C. Simmons (Boston):

SUMMARY

While many reports of the end-results of operations for the cure of cancer have been published, and while certain standards such as the arbitrary three or five year limit of time are generally accepted, no uniform plan for the reporting of surgical statistics exists, so that it is practically impossible to compare the results from one clinic with those of another. This matter first attracted our attention in 1907, when we reported the end-results of operations for cancer of the breast at the Massachusetts General Hospital, and again in 1908, when we made a study of the end-results of operations of cancer of the mouth, tongue, and jaw. In the latter report the following statements were made:

A comparison of these statistics with those of other writers was made and a large number of statistical papers were consulted. It was found, however, that the varying conditions under which the statistics were prepared made a comparison difficult without injustice to one or another writer. *It would seem that a generally accepted standard form for the report of end-results of operations for cancer should be secured.*

In a recent paper on cancer of the breast an arbitrary standard was adopted, and that standard has been maintained in the present communication. It is, briefly, as follows:

1. A definite period of time has been selected ending at least three years prior to the report, and *all* of the cases entered in the hospital records under the given diagnosis have been investigated.
2. No case has been accepted as cancer without proof by pathological examination, or subsequent recurrence, or autopsy.
3. Cases which have survived, at last report, only a portion of the necessary three-year period, are eliminated as inconclusive.
4. Cases not traced at all after discharge from the hospital, and not appearing in the mortality statistics of their place of residence, are eliminated as inconclusive.
5. All cases fulfilling the above requirements are published and counted in the statistics with such subdivision into radical and palliative operations as may seem expedient.

It is the hope of the writers that this standard requirement may be adopted by others for similar reports. A writer who counts as successful cases those which have survived a period of less than the arbitrary three-year standard, or who accepts the clinical diagnosis of cancer without microscopic examination of the specimen, cannot in justice ask to have his statistics accepted for comparison with those of surgeons who exercise a more careful scrutiny. It is well said by Halsted: "It is especially true of breast cancer that the surgeon interested in furnishing the best statistics may in perfectly honorable ways provide them."

The obvious solution of this difficulty is to publish *all* cases which fulfill the present conditions and allow the reader to draw his own conclusions. If one surgeon reports 100 cases, of which 25 are palliative and 75 radical operations, his statistics for radical operations may well be expected to be inferior to those of the surgeon who performs 50 palliative and 50 radical operations in the same total number of cases. Without these figures, however, the opportunity for comparison is lost, and the two sets of statistics apparently meet on equal terms.

Since the method above described has proved satisfactory in operation, the writers would again present it as a standard suitable for adoption by the profession at large; and as a further example of its value they would here report, briefly, a second series of cases of cancer of the breast which were operated upon at the Massachusetts General Hospital between the dates of August 1, 1911, and April 1, 1914. During this period the policy of special assignments of cases to different members of the staff was in operation, and to the writers were given all cases of diseases of the breast which entered the hospital during that time. By following the same plan of reporting adopted in the earlier series a direct comparison of the work in the two series of cases is made possible. The only discrepancy lies in the fact that a five year period of time was adopted in the second series while the three year period was employed, as was customary at that time, in the earlier cases.

The scheme for the reporting may be summarized as follows:

- A. Record all cases entering surgical wards with the specified diagnosis during period selected.
- B. Eliminate all re-entries. (No single case should appear twice in the report).
- C. Eliminate all cases recurrent after previous operation in hospital or elsewhere; these are not cases of primary attempt to cure.
- D. Deducting B+C from A we have the number of cases of cancer available for study of operability, mortality, and other operative statistics. These cases may then be subdivided as follows:
 - E. Cases of radical operation.
 - F. Cases of palliative operation.
 - G. No operation advised or performed.
 - H. Operative deaths.
 - I. Operative mortality $H+E+F$.
 - J. Operability (radical operations) $E+D$.
 - K. Operability (all operations) $E+F+D$.

For the study of end-results of treatment certain cases included in D are of no value and should be deducted, viz:

 - L. Cases not proved to be cancer either by pathological examination of tissue, or recurrence, or autopsy.
 - M. Cases untraced after leaving hospital for required interval of time—three years, five years.
 - N. Cases that have died of other diseases within the required interval of time, and without evidence of recurrence.
- O. The cases remaining after deducting L, M, and N, from D, are available for study of end-results, as follows:
 - P. Radical operations.
 - Q. Palliative operations.
 - R. No operation.
 - S. Number of cases alive without recurrence (3 years, 5 years).
 - T. Number of cases died (over 3 years or over 5 years) without recurrence.
 - U. Number of 3 year or 5 year "cures": all operations.
 - V. Number of 3 year or 5 year "cures": radical operations.
 - W. Percentage of 3 year or 5 year "cures": all operations ($U+P+Q$).
 - X. Percentage of 3 year or 5 year "cures": radical operations ($V+P$).

End-results: Carcinoma of the breast

	1894-1904	1911-1914
A. Total entries. Carcinoma Breast.....	613	115
B. Re-entries (entered more than once).....	80	8
C. Recurrence from previous operation.....	65	4

D. Cases available for study of operability, mortality, etc.....	468	103
E. Radical operation.....	360	74
F. Palliative operation.....	56	20
G. No operation.....	52	9
H. Operative deaths.....	15	0
I. Operative mortality (H+E+F).....	3.6%	0
J. Operability: Radical operations (E+D).....	77%	72%
K. Operability: All operations (E+F+D).....	89%	91%
L. Inconclusive cases; Lack pathological examination.....		0
M. Inconclusive cases: Untraced.....	38	5
N. Inconclusive cases: Died within time limit...	2	3
O. Cases available for end-result data.....	428	95
P. Radical operations.....	320	69
Q. Palliative operations.....	56	17
R. No operation.....	52	9
S. No. cases alive and well.....	64	22
T. No. cases died without recurrence.....	7	1
U. No. 5 year "cures" (all operations).....	71	23
V. No. 5 year "cures" (radical operations).....	67	22
W. Percentage of "cures" (all operations) (U+P+Q).....	19%	27%
X. Percentage of "cures" (radical operations) (V+P).....	21%	32%

This paper appears in full in the Boston Med. & Surg. Jour. 1921, clxxxv, 253.

DISCUSSION

Dr. Bell (Minneapolis): Very important considerations in the curability of carcinoma of the breast are the size of the tumor, regardless of its histologic type, and the presence of metastases in the axillary nodes at the time of the operation. Has Dr. Simmons any information on the cured cases relative to these points?

Dr. Simmons: We divided the cases clinically into four groups. In group 1 were placed cases having a small tumor and no palpable axillary nodes. Seventy-one per cent of these cases are living without recurrence. Group 2 comprised slightly more advanced cases with small palpable axillary nodes. Thirty-three per cent of the cases in this group are living. In group 3 were placed the advanced cases, with many axillary nodes, in which an attempt at cure by radical operation was made. Ten per cent of the cases in this group are living. Group 4 consisted of advanced cases in which a palliative operation only was attempted. Five per cent of this group, one case, are living.

There were two cases of colloid carcinoma in both of which there were axillary metastases. These cases are both living without recurrence.

Infection of the axillary nodes, as shown by the microscopic examination, had a distinct bearing on the prognosis of the case. Thus there were twenty-four per cent cures in the cases showing axillary infection, and fifty-six per cent cures in those where the nodes were not involved.

It is interesting to note that in sixty-nine per cent of the cases the radical operation did all that could be expected of it; that is, if the patient died she died from remote metastases and without recurrence in the scar or axilla.

10. RADIUM IN CANCER OF THE BLADDER

Dr. George Gilbert Smith (Boston):

SUMMARY

A report of experiences at the Huntington Memorial Hospital, Boston, with the use of radium in 24 cases of cancer of the bladder. Of these cases only 1 might have been suitable for radical removal. Seven cases were quoted to show the effect of single applications of radium in the effort to determine a standard dosage. Fifteen cases were treated by the introduction of screened radium emanation into the bladder cavity. Of these 9 cases had a total treatment of 600 mc. hours or more. Analysis of these cases showed that while 2 of them gave a temporary diminution in the amount of tumor and 1 possibly showed a total destruction of carcinoma, none was completely cured even clinically. Six cases were treated by the introduction of bare emanation tubes into the tumor itself. In 3 cases this was done without opening the bladder; in 3 it was done by cystotomy. Of these cases 1 appeared to be completely cured after 1 year. Another case dying six weeks after operation showed no carcinoma at autopsy. With the other cases insufficient time has elapsed to determine the result of the treatment.

Conclusions

1. It is useless to attempt to cure with radium infiltrating carcinoma, which involve large portions of the bladder wall. Necrosis of the bladder will be brought about by any dosage which will materially influence the tumor.
2. Certain superficial cancers of the bladder may be reduced in extent by the application of screened radium emanation to their surface. This may occur without necrosis of the bladder wall.
3. To accomplish this effect, 400 mc. hours, with screening of 0.5 mm. silver, applied not oftener than once in six weeks, has been successful, and has not caused any considerable reaction in the bladder.
4. The greatest effect is produced by the first 3 or 4 applications of radium.
5. If the tumor begins to grow again, further radium applications have little deterrent effect.
6. The best way to employ radium in cancer of the bladder is by the implantation of bare emanation tubes in the tumor, allowing one tube to each cubic centimeter. Steel needles containing radium may be employed in the same way, except that they must be withdrawn after adequate exposure has been made.

7. The necrosis caused by the implantation of radium in bladder tumors persists for at least three months.

DISCUSSION

Dr. Wood: I think that these papers by Dr. Smith and Dr. Simmons are admirable. It is important that we laboratory men check up our results on human beings; we care nothing for rats and mice in themselves. One interesting point is the statement that a radiated tumor is resistant after scar tissue has been formed. The observation has been made by others also. I can not think that the carcinoma cell undergoes any change, simply because of the presence of scar tissue. The radium dosage for cancer cells in tissues not highly vascular, or in contact with normal tissue, is very much higher than for the same cancer cells where thrombosis can take place and cause extensive destruction. For mouse cancer cells, the dose is 2400 mc. hours at a distance of two centimeters; you see how resistant the cell is. Now Dr. Simmons has shown that 630 mc. hours are all that can be allowed, so that we are still far from able to guarantee killing the cell, or from promising that other cells will in any way affect the life of the cancer cell. I do not believe, for example, that lymphocytes or connective tissue cells destroy cancer cells; there is not the slightest evidence to that effect. Indeed, the cancer cell may lie quiescent in the connective tissue for seven, ten, or fifteen years.

11. PROBLEMS IN CANCER RESEARCH

Dr. Montrose Burrows (Saint Louis):

(Published in the JOURNAL OF CANCER RESEARCH, 1921, VI, 131)

DISCUSSION

Dr. Burrows: In reference to the discussion on the alkalinity of the blood in cancer patients, I wish to state that we have found no direct correlation between the alkalosis in many cancer patients and the anaemia which is invariably present. Alkalosis in anaemic patients is a well established fact. So far our conclusions in relation to anaemia have been drawn from a comparison of the blood tests for pH which we have had made and the blood picture recorded on the hospital history. We are now anxious to make all these tests for anaemia ourselves. We also feel sure that other conditions such as lesions of the pancreas and kidneys will alter materially the pH determinations of the blood in the cancer patients. Certain of our cases as well as others cited in the literature have indicated this fact.

In referring to Dr. Clowes's statement about a lipoid membrane, I wish to call his attention to the fact that such exists in the tissue culture. We noted this fact in the literature in an article on "The cultivation of bladder and prostatic tumors" (Jour. Urol., vol. 1, no. 1). In analyzing the property of stereotropism introduced for body cells by Harrison,

we found that these cells were not in contact with the cover glass or free surface of the medium as Harrison stated, but separated from the surfaces by a surface film of substance which is insoluble in the medium. This was particularly easy to demonstrate in the cultures of bladder and prostatic carcinomata.

Returning to the question of blood alkalosis in cancer, our work has further suggested to us that the alkalosis is due to the addition of an alkaline substance from the tumor into the blood. The pH becomes changed because of the lack of a very active compensating mechanism for alkalies (Bayliss).

The demonstration of an alkalosis in the plant cancers and in the culture of the *Bacillus tumefaciens* gives further confirmation of this fact.

12. MASSAGE AND METASTASIS

Dr. L. C. Knox (New York, by invitation): Presented by *Dr. F. C. Wood*:

SUMMARY

The general conditions underlying the formation of metastatic deposits from malignant tumors have long been of interest to the surgeon as determining the scope and direction of his operative procedures and even the feasibility of operation.

In this country, although the dermatologists have always made biopsies in doubtful cases, there has been during recent years a great deal of discussion among surgeons as to the danger of incising tumors. Isolated instances of apparent distribution following diagnostic incisions have led to the widespread feeling that such incisions tend to distribute the tumor. This attitude, strangely enough, has existed only in the United States; the English, French, and German surgeons do not seem to have considered the matter as of importance.

On the other hand, the relationship of massage to the production of tumors has excited but little interest in the minds of the practitioners of surgery and there are but few recorded clinical observations of the spreading of tumors by massage, though such distribution takes place very frequently under the administration of the mechanical treatment used by the osteopath and the chiropractor to "disperse" tumors. Some of the most extraordinary instances of widespread metastatic involvement of the entire body have been seen following massage of a carcinoma of the breast. Several instances, in which very extensive and early metastasis occurred after small tumors had been repeatedly examined and rather firmly squeezed or handled by the physician making the examination, called the writer's attention to the importance of this phase of the metastasis question.

Though the problem is one easily attacked from an experimental point of view, but little work has been done on it, with the exception of experiments by *Dr. E. E. Tyzzer*, who some years ago observed that massage of a mouse tumor considerably increased the number of metas-

tases. His experiments were confined to only one type of tumor; and, therefore, it seemed of great practical and scientific interest to extend, if possible, his observations to a large variety of tumors of different histological forms so as to correlate the results obtained from animals with those observed in human beings.

It is obvious that a small-cell tumor in which the cells are closely related to the blood-vessels, such as the lymphosarcomata, would metastasize early and extensively, while the firmer fibrosarcomata might be expected to offer considerable resistance to the removal of tumor particles into the lymph-channels and the blood-vessels. As the lymphatic system in animals is not as extensive as that in man, metastasis is most frequent by way of the blood-vessels; hence, the tumor cells reach the lungs first, and the effect of massage would be expected to increase very greatly the number of secondary tumors in these organs. This was found to be the case.

Two series of experiments were carried out with the same technique, one two years after the other. The first consisted in the inoculation of mice and rats with six strains of carcinomata and four strains of sarcomata. About 500 animals survived for the completion of the experiment. As soon as the tumors were palpable, half of them were massaged for thirty seconds on alternate days for about two weeks; the other half were used for controls. After this, the tumors were removed by operation, and the animals were killed at the end of thirty days. The lungs of these animals, as well as those of the animals which died spontaneously during the course of the experiment, were examined microscopically, and the number of metastases and emboli was noted. In the second experiment only one strain, a polyhedral-cell sarcoma, was used with 50 mice. The results of the two experiments were approximately the same.

It was found that in all but three strains there was a larger percentage of lung emboli in the massaged mice than in the controls; that there was also usually a higher percentage of actively growing metastases; and that the total number of metastatic particles of both quiescent emboli and growing metastases constantly increased, the variation being from 1 per cent to 37 per cent. In this respect, little difference was found between the polyhedral-cell sarcomata and the carcinomata. It was found, also, that fibrosarcomata are not so apt to metastasize when manipulated as are the other types.

13. FURTHER EXPERIMENTS ON SENSITIZATION TO HEAT BY RADIATION

Dr. William T. Bovie (Boston):

SUMMARY

The experiments reported in this paper concern the changes which take place between the time of radiation and the appearance of the first visible effects.

At a previous meeting I have discussed the rate of recovery of *Paramecium* from the destructive action of fluorite rays, and the sensitization to heat resulting from the exposure to fluorite rays.

My interpretation of the results of these investigations was incorrect, because through faulty methods of experimentation the effects of ozone formed by the light were not excluded.

The experiments reported in the present communication were conducted in such a manner as to exclude the effects of ozone.

A very definite sensitization to heat was demonstrated.

By increasing the temperature of the organism after the radiation, the length of the latent period is shortened. All of the changes produced by the radiation are intensified and the total number of deaths for a constant dosage is increased.

The effect is greater the higher the temperature and the longer the time during which the organism is maintained at the increased temperature.

The temperatures used were not high enough to affect normal unirradiated organisms, nor were the effects to be observed if the organisms were subjected to the increased temperature before the exposure to the rays.

DISCUSSION

Dr. Clowes: At what temperature do these changes start?

Dr. Bovie: Room temperature, 22°C.

Dr. Clowes: You must take into consideration variation in temperature, for radiation is ultimately chemical.

Dr. Bovie: We have a thermo-couple connected with the slide, and make certain that the temperature is back at normal before we radiate. It would not make a great difference, however, because light reactions are not influenced by changes in temperature.

Dr. Wood: It may interest Dr. Bovie to know that we are not able to detect any difference in the cancer cells between heating first and radiating afterward, or radiating first and heating afterward. The point we used was the death point determined by animal inoculation. This is far from what he is working with. We can observe it accurately. We get an approximation to a logarithmic curve but whether this is the true form of the curve is doubtful.

14. MALIGNANT TUMORS OF THE THYROID

Dr. Louis B. Wilson (Rochester, Minn.):

SUMMARY

This paper presents an analysis of the pathological data concerning 290 patients with malignant tumors of the thyroid examined in the

Mayo Clinic between January 1, 1901, and January 1, 1921. Lantern slides illustrating the various histologic types of tumor were shown and the clinical course of the disease in cases of the various types discussed in connection therewith. The following is a summary of the principal points in the paper:

1. Malignant tumors of the thyroid are much more frequent than is generally believed. Correct clinical diagnosis is frequently missed, (a) because they may have periods of development of from five to fifteen years and patients are not followed up long enough after operation; and (b) because not infrequently the tumor is relatively small and the character of metastasis is not determined, owing to the rarity of necropsies.

2. Pathologic diagnosis is difficult owing to the great variation in the histology of the tumor and its resemblance to that of non-malignant processes.

3. There has been a marked failure of American surgeons to report their cases of malignant tumors of the thyroid; this should be corrected.

4. Sufficient observations are not at hand for determining the geographic incidence.

5. The age incidence at the date of diagnosis is greatest in the fifth decade.

6. The distribution by sex is about one man to two women.

7. Patients usually seek medical advice on the occasion of recent rapid growth in a long standing nodular tumor of the thyroid. Some give histories of slow continuous growth.

8. Early thorough operation gives a fair percentage of cures. Palliative operation in late cases with extensive local involvement is warranted.

9. Pathologic diagnosis must take into account the usual development of malignant tumors of the thyroid from proliferating embryonic adenomas.

10. The pathologist must be thoroughly familiar with the characteristics of proliferating adenomas (as first described by Langhans) in all their stages.

11. The pathologist must be on the lookout for a possible relationship between bizarre metastatic growths and tumors of the thyroid.

12. The pathologist, in his diagnosis for the guidance of the surgeon, must consider the relative preponderance of proliferative and degenerative processes in the tumor; but a proliferating adenoma in a patient of cancer age should not be considered benign unless the process of degeneration is very extensive and thoroughly overbalances that of proliferation.

DISCUSSION

Dr. Wells: My experience in the Chicago district corroborates Dr. Wilson's statements; there, also, tumors of the thyroid are not uncommon. It is hard to find a normal thyroid in experimental dogs in

Chicago, and I have many sent to me with carcinoma of the thyroid. There have been but a few definite tumors of the thyroid in mice in the Slye stock.

15. EXPERIMENTAL PRODUCTION OF TUMORS

Dr. F. C. Wood:

SUMMARY

Dr. Wood reported the experimental production of tumors in the Crocker Laboratory by Dr. Frederick Bullock and Miss M. R. Curtis. The method employed was to feed rats with ova from cat feces. The ova were those of the *Tenia crassicolis*, a frequent inhabitant of the cat intestine.

The organism penetrates the mucous membrane of the intestine, passes to the liver and there forms a cyst. In the wall of such cysts, single or multiple sarcomata develop after a period which is never less than eight months. These sarcomata are of two main types, spindle-cell and large polyhedral-cell. They are highly malignant, metastasizing throughout the animals, and are transplantable, giving a high percentage of successful transplants at the first inoculation.

The importance of the discovery is the large yield of tumors and ease of the method, in contrast with the laborious tar painting process. One strain of animals gave approximately 50 per cent of tumors among all rats infected.

DISCUSSION

Dr. Bell: Has Dr. Wood tried any filtration experiments?

Dr. Wood: Some years ago large series of filtrations were carried out on various tumors in the Crocker Laboratory but without success.

Dr. Bell: I would like to ask whether the blood shows any changes in these very malignant sarcomata? Does it suggest a leukemia?

Dr. Wood: No changes at all.

FURTHER INVESTIGATIONS ON THE ORIGIN OF TUMORS IN MICE

VII. TUMOR AGE AND TUMOR INCIDENCE

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1. In our former publications we have analysed the relation between tumor age and tumor rate in the various strains of mice (1). We found that in those strains in which the tumor rate was high the tumors tended to appear at an earlier period of life than in strains in which the tumor rate was lower. In addition, we found indications that there existed a special tumor age in certain strains of mice. In general cancer age was transmitted by heredity as well as cancer rate.

The present communication differs from our preceding ones in the following respects: (a) We analyse here our total material connectedly, while previously we considered only certain parts. (b) The method of computation of the relations between tumor age and tumor rate, which we used formerly, was complicated and made a comparison between different strains and groups of mice difficult. We now make use of a simpler way of figuring out the relation between tumor age and tumor rate and thus are enabled to compare more readily the different strains. (c) We extend our analysis in various directions and explain our results more fully on the basis of multiple factors.

2. In order to determine the tumor age of a certain family strain or group of strains we proceeded in the following manner. We determined the number of mice alive in the beginning of each of the three age periods (I age period, 7-12 months; II age period, 13-17 months; III age period, 18 months and older.) We

then determined the number of mice which developed tumors in each of these age periods and figured out what percentage of mice were in each age period affected by cancer. These percentage figures we then reduced to a basis of 100 per cent cancer incidence, in order to obtain figures for the cancer age in different strains which were independent of the absolute number of tumor mice in each strain. We may give as an example of this mode of determination the figures obtained for the total of all $8\frac{1}{2} + 328$ strains; the last figure on each line indicates the number of mice in which tumors developed in each age period in a unit of 100 tumor mice. The figure 56.4 per cent indicates the tumor incidence of the whole strain.

		$8\frac{1}{2} + 328$			
I age period.	566 mice	194 tumor mice = 34%	27%		
II age period.	208 mice	117 tumor mice = 56%	44%	56.4%	
III age period.	24 mice	9 tumor mice = 38%	29%		

3. We divide all our strains into high, medium, and low tumor rate strains. The high tumor rate strains have an incidence of more than 40 per cent. The medium tumor rate strains have an incidence varying between 20 and 40 per cent, and the low tumor rate strains have an incidence below 20 per cent.

In our lists we state first the name of the strain, then we give the number of mice and tumor rate in each strain and in the last three rows the tumor incidence in each age period, reduced to a unit of 100 tumor mice. Added in brackets to these figures are in certain strains the absolute percentage figures of tumors in each age period. Wherever the name of the strain as a whole is in brackets, the figures were merely given in the list; they were for obvious reasons not used in figuring out the incidence in each age period in all of the strains belonging to one of the classes (high, medium, low rate tumor mice).

If we compare the figures giving the percentage of mice affected by cancer in the three age periods, we may draw the following conclusions: (1) If the number of mice in a group is small, for instance, below 50, or even somewhat higher, the percentage age figures are no longer of value; if the number of mice exceeds one hundred, the percentage figures begin to be much more trust-

High rate tumor mice

STRAIN	NUM- BER OF MICE	TUMOR RATE	PERCENTAGE OF TUMORS IN		
			I age period	II age period	III age period
		per cent	per cent	per cent	per cent
(London Blue and White).....	31	55.0	18.0 (26.0)	37.0 (54.0)	45.0 (66.0)
Total 8½ + 328.....	566	56.4	27.0 (34.0)	44.0 (56.0)	29.0 (38.0)
(8½ + 328 A).....	118	51.0	29.0 (34.0)	42.6 (50.0)	28.4 (33.3)
(8½ + 328 B).....	226	56.0	25.4 (32.0)	41.0 (52.5)	33.6 (42.0)
[8½ + 328 (new cross)].....	146	57.5	26.5	44.0	29.5
[782a (= 8½ + 328)].....	76	60.0	35.0 (38.0)	65.0 (70.0)	0.0 (0)
(English).....	24	46.0	25.0 (29.0)	32.0 (37.0)	43.0 (50.0)
(English A).....	175	63.0	20.0 (29.0)	45.0 (64.0)	35.0 (50.0)
[Family 101 (English)].....	99	71.0	21.0 (45.5)	32.0 (69.5)	47.0 (100)
(English Sable A).....	252	70.0	28.0 (46.0)	34.0 (54.5)	38.0 (62.0)
(English Sable B).....	48	67.0	20.0 (27.0)	49.0 (65.0)	31.0 (40.0)
[Family 437 (English Sable)]...	55	82.0	37.0 (58.0)	63.0 (100)	0.0
(Smaller English Family).....	36	56.0	46.0 (42.0)	54.0 (50.0)	0.0
[344 + 328 and (344 + 328) + 437] English Sable.....	34	79.0	46.0	54.0	0.0
Total English.....	689	67.6	25.0 (40.6)	39.0 (62.5)	36.0 (54.5)
[(European 151 + Id of No.10) + 101 (English) Total].....	62	42.0	25.0 (26.0)	36.0 (37.0)	39.0 (40.0)
[(European 151 + Id of No.10) + 101 English A].....	27	55.0	40.0	60.0	0.0
" (English B).....	35	31.0	17.7	24.0	58.3
Total Michigan Wild + English 101.....	70	48.0	21.0 (23.0)	28.0 (31.0)	51.0 (57.0)
(English A).....	50	58.0	19.0	29.0	52.0
(English B).....	20	25.0	29.5	21.5	49.0
[European 151 + Id of No. 10 (November 3)].....	254	72.0	15.0 (25.0)	33.0 (55.0)	52.0 (88.0)
Total European 151 + IId of No. 10 (November 8).....	187	54.5	15.0	36.0	49.0
(Total European A).....	96	65.0	14.0	36.0	50.0
(Total European B).....	91	44.0	17.5	33.5	49.0
(8½ + IId of No. 10) A.....	82	49.0	9.0	35.0	56.0
English Sable + [(European + 103) F ₁ + IIIId of No. 10] F ₂	6	83.0	0.0	38.0	62.0
European + English Tan (daughter of tumor mouse 146 B).....	33	42.0	4.6	57.5	37.9
(Silver + 10 B).....	11	64.0	17.0	20.0	63.0
Total English Tan + German. (English Tan + German A)...	60	46.6	24.0	41.0	35.0
(English Tan + German B)...	44	49.0	21.0	35.0	44.0
(English Tan + German B)...	21	43.0	33.0	67.0	0.0

STRAIN	NUM- BER OF MICE	TUMOR RATE	PERCENTAGE OF TUMORS IN		
			I age period	II age period	III age period
			per cent	per cent	per cent
Unknown (probably English)	32	72.0	30.0	36.0	34.0
Total 198 English + (8 + German).....	217	54.0	20.0 (28.0)	41.0 (58.0)	39.0 (55.0)
[198 English + (8 + German A)]	82	63.0	31.0	69.0	0.0
[198 English + (8 + German B)]	135	49.0	18.5	35.0	46.5
(German A).....	20	50.0	12.0	36.0	52.0
(8 + German A).....	244	41.0	13.0	40.0	47.0
[Family 240 of (8 + German)]	14	43.0	29.0	33.0	0.0
Combined (Silver + English A) and Silver + (English Sable C).....	40	57.5	23.0	57.0	20.0
English Sable 344 + German..	45	64.5	20.5	26.0	53.5
English Sable + (English Silver + Id of No. 10).....	23	65.0	23.0	77.0	0.0
English Sable + (European + Id No. 10).....	125	55.0	25.0	40.0	35.0
(English Sable + Cream Y)..	68	53.0	14.0	46.0	40.0
693 (= English Tan + Cream) + 773 (= 8½ + 328).....	35	60.0	38.0	62.0	0.0
794 (German + Carter) + (8 + German).....	90	49.0	18.0	25.0	57.0
794 (German + Carter) + [198 (English) + (No. 8 + German) F ₁].....	37	61.0	21.0	26.0	53.0
Waltzer + No. 8.....	52	46.0	20.5	31.5	48.0
240 (8 + German) + Cream...	38	76.0	11.0	37.0	52.0
8½ + English Sable.....	48	61.0	40.6	59.0	0.0
Waltzer + English Orange and combined cross.....	65	65.0	36.0	64.0	0.0

worthy. (2) With this restriction as to numbers, we may conclude that the age distribution of tumors is at least as characteristic of strains and serviceable for their distinction as the tumor incidence; in fact in certain cases it is a finer instrument for the characterization of strains than the tumor rate. The tumor rate of two strains may be similar, but the tumor age may allow a differentiation between the two strains.

Thus if we consider especially the high tumor rate strains we notice that the tumor rate of European 151 and I daughter of No. 10 and of European 151 and II daughter of No. 10 on the one hand and of $8\frac{1}{2}$ + 328 and English and its substrains on the other hand do not differ very much, while the tumor age is quite distinct.

The percentage figures for the first and third age period are similar in the former two strains and differ very much from the figures in the latter strains. There is even a difference between English and $8\frac{1}{2}$ + 328, both very high rate tumor strains; in the English the tumors appear somewhat later than in the $8\frac{1}{2}$ + 328 strain. This comes out even more clearly if we consider the percentage figures for the third age period than those for the first age period.

4. If we compare the tumor ages of the substrains with those of the main strains we find that on the whole they agree provided the figures used are not too small. This applies for instance to the English, $8\frac{1}{2}$ + 328 and European + I or II daughter of No. 10 and their substrains.

In the case of the strain "unknown" there was some indication that it was a substrain of the English. Not only the tumor rate, but also the tumor age of this strain furnish confirmatory evidence for this conclusion.

5. In crosses between English and the majority of other strains in which the tumor incidence is high the tumor age is similar to that of the English. $8\frac{1}{2}$ + 328 is a cross of the female offspring of a particular English tumor mouse with an $8\frac{1}{2}$ male. Here we have, owing to the influence of the English mother, a very early tumor age which exceeded even the average age of the English mice. On the whole, we find the English or a similar tumor age in the following additional crosses: (European 151 + I daughter of No. 10) + 101 English, Michigan Wild + English 101. (European 151 + I daughter of No. 10) + 101 English) A. English Tan + German, 198 English + (8 + German), Silver + English, English Sable + German English Sable + (English Silver + I daughter of No. 10), English Sable + (European + I daughter of No. 10) (English Tan + Cream) + ($8\frac{1}{2}$ + 328),

Medium rate tumor mice

STRAIN	NUM- BER OF MICE	TUMOR RATE	PERCENTAGE OF TUMORS IN			
			I age period	II age period	III age period	
		per cent	per cent	per cent	per cent	
(Complete English-Cream Hybrids).....	1057	24.3	20.0 (8.6)	42.0 (18.0)	38.0 (16.1)	
English-Cream Hybrids, medium rate.....	739	32.3	19.0 (11.3)	41.0 (24.4)	40.0 (24.0)	
(English Sable 344 + Black Cream).....	92	23.0	18.5	40.0	41.5	
(Black Cream + English White).....	143	39.0	19.0	47.0	34.0	
(Cream + English, October, 1913).....	109	23.0	12.5	31.0	56.5	
(White Cream + White English).....	27	29.0	36.0	26.0	38.0	
[Cream-English (Descendants of English Sable 1031)].....	26	31.0	6.9	44.6	48.5	
(English Sable + Cream Y, high rate).....	68	53.0	14.0	46.0	40.0	
(693 + Cream).....	7	28.5				
[Total English Tan (121) + Cream].....	267	31.0	22.0 (12.0)	38.0 (21.0)	40.0 (22.0)	
Total London.....	452	28.0	22.0 (10.9)	45.0 (22.0)	33.0 (16.0)	
(London A).....	120	27.0	13.0 (7.5)	36.0 (19.0)	51.0 (27.0)	
(London B).....	61	38.0	29.0 (16.0)	56.5 (32.0)	15.8 (8.0)	
(London C).....	197	28.0	11.0 (25.5)	49.0 (21.0)	25.5 (11.0)	
(London 481).....	43	0.0				
(London Blue and White).....	31	55.0	18.0	37.0	45.0	
Total European + 8 F ₁	151	28.0	12.5 (6.0)	44.25 (21)	44.25 (21)	
(European + 8 F ₁ A).....	125	30.0	12.5	48.0	39.5	
(European + 8 F ₁ B).....	26	23.0	9.5	26.0	64.5	
Total Heitler.....	196	25.0	12.5 (61.0)	64.0 (31.0)	23.5 (11.5)	
(Heitler A).....	102	27.0	8.5 (4.0)	91.5 (43.0)	0.0 (0.0)	
(Heitler B).....	94	22.3	19.0 (8.5)	39.0 (18.0)	42.0 (19.0)	
101 (English) + (European + 103).....	152	34.0	5.5 (4.0)	39.5 (29.0)	55.0 (40.0)	
Total European + English Tan.....	109	32.1	7.4 (4.0)	50.0 (27.0)	42.6 (23.0)	
(European + English Tan A).....	76	28.0	4.0	45.0	51.0	
(European + English Tan B).....	33	42.0	4.6	57.5	37.9	
(June, 1914, Family of Cream B).....	40	35.0	4.6	33.5	61.9	
Total No. 8.....	243	27.5	12.0 (7.0)	27.5 (16.0)	60.6 (35.0)	
(No. 8 A).....	213	30.0	11.4 (7.1)	32.2 (19.0)	56.4 (36.0)	
No. 8 A ₁	145	34.0	17.0 (11.0)	33.0 (21.5)	50.0 (32.0)	
(No. 8 A ₂).....	68	22.0	0.0 (0.0)	24.5 (15.4)	75.5 (47.3)	
(No. 8 B).....	30	10.0	0.0 (0.0)	0.0 (0.0)	100 (20.0)	
Carter.....	67	39.0	26.0	32.0	42.0	

STRAIN	NUM- BER OF MICE	TUMOR RATE	PERCENTAGE OF TUMORS IN		
			I age period	II age period	III age period
		per cent	per cent	per cent	per cent
Total 8½ + II daughter of No. 10.....	110	40.0	8.5	40.0	51.5
(8½ + II daughter of No. 10 A).....	82	49.0	9.0	35.0	56.0
(8½ + II daughter of No. 10 B).....	28	14.0	0.0	100	0.0
(8½ + II daughter of No. 10) + Id of No. 10.....	72	36.0	8.9	20.8	70.3
(European 151 + Id of No. 10) + 101 English B.....	35	31.0	17.7	24.0	58.3
Total Silver + Id of No. 10....	245	37.0	17.0	27.0	56.0
(Silver + 10 A).....	234	36.0	17.0	26.0	57.0
(Silver + 10 B).....	11	64.0	17.0	20.0	63.0
Total medium rate Cream + Id of No. 10.....	370	34.0	10.0 (5.4)	32.0 (17.0)	58.0 (31.0)
(Total Cream + 10 includes one low rate cross).....	498	27.5	8.4	29.0	62.6
(Cream + 10 A).....	174	36.0	5.5	28.0	66.5
(Cream + 10 B).....	47	26.0	22.0	46.0	32.0
(White Cream + Id of No. 10) (Michigan Wild + English 101 B).....	149	34.0	12.0 (8.0)	33.0 (20.0)	55.0 (38.0)
.....	20	25.0	29.5	21.5	49.0
Total German.....	42	40.5	15.5 (19.0)	30.3 (37.0)	54.2 (66.0)
(German A).....	20	50.0	12.0	36.0	52.0
(German B).....	22	32.0	23.0	51.0	0.0
Total 8 + German.....	373	34.0	14.0 (8.0)	42.0 (24.0)	44.0 (25.0)
(8 + German A).....	244	41.0	13.0	40.0	47.0
(8 + German B).....	129	21.0	23.0	51.0	26.0
(Family 240 = 8 + German)...	14	43.0	47.0	53.0	0.0
German + Carter B.....	113	33.5	22.0	31.0	47.0
794 = German + Carter.....	25	36.0	5.0	29.0	66.0
English (344) + 8½ F ₄ and F ₅ ..	155	25.0	21.0	39.5	39.5
Cream + [198 (English) + (8 + German) F ₄].....	33	33.5	36.5 (15.0)	73.5 (26.0)	0.0 (0.0)
Black Cream + European....	104	32.0	11.0	32.0	57.0
European + Cream.....	25	28.0	5.3	49.0	65.7
(Waltzer + White English) + (Cream + 10).....	19	21.0	18.9	81.1	0.0
Vermont Wild + English F ₁ ...	373	36.3	11.0	30.0	59.0
(½ Waltzer ½ Cream) + No. 8..	32	31.0	28.6	46.4	25.0
German + No. 6 F ₄	52	37.0	11.9	24.0	64.1
Waltzer + White English and (Waltzer + White English) + English.....	99	26.0	29.0	28.0	43.0
(Cream B June 1914 Family)...	40	35.0	4.6	33.5	61.9

German + Carter + (198 (English) + (No. 8 + German) F₃), 8½ + English Sable, Waltzer + English Orange. Waltzer No. 8 is similar. It is different, however, in a cross between English and Cream which approached the tumor rate of the English, namely, English Sable + Cream Y. The tumor rate is here 55 per cent, but the tumor age is distinctly different; it is intermediate between that of the English and Cream.

German, 8 + German, German + Carter have a later tumor age; the same applies to the crosses between these strains. Somewhat later even is the tumor age of (8 + German) + Cream, owing to the influence of the Cream; yet the influence of the 8 + German causes the tumor age to be earlier than that of the Cream. In a similar way the tumor age of the (8½ + II daughter of No. 10) A is later, although in this group the tumor rate is high.

MEDIUM RATE TUMOR MICE

6. While in the large majority of the hybrids between Cream and English the tumor rate is intermediate (32.3 per cent) between that of the Cream and English strain, in some of them it is low like that of the Cream, and in one of them, as we have seen, it approaches that of the English strain. We have divided these hybrids in two larger groups, one containing the low and the other the medium tumor rate strains. We find that the tumor age in both reaches almost, but not quite, that of the English parent. Tumors appear relatively early in these groups; only in two of these hybrid strains the tumor age is intermediate. Again we notice that in small strains comprising only 26 or 27 individuals the determination of the tumor age is no longer certain.

7. The result as to the effect of hybridization on tumor age is different in another hybrid strain in which Cream was crossed with the No. 10 (I daughter) strain. While in this case the tumor rate is intermediate, the tumor age approaches that of the Cream, although it does not quite reach the lateness of the tumor age of the Cream. On the other hand, in a cross between Silver and the same No. 10 strain, although the tumor rate is

again intermediate—in accordance with the low tumor rate of Silver which almost corresponds to that of the Cream—the tumor age is decidedly different and very much resembles that of the No. 10 strain; this is in accordance with the fact that, as far as can be determined from the restricted number of Silver mice at our disposal, the tumor age is in this latter strain very much higher than that of the Cream.

This dissociation between tumor age and tumor rate is likewise apparent in the London strain, where the tumor age almost reaches that of the English, while the tumor rate is very much lower.

A similar dissociation we find in the following strains: Carter (German + Carter) B, English (344) + $8\frac{1}{2}$, Cream + (198 English + (8 + German) F₄) and in the Waltzer and English as well as the Waltzer and Cream hybrids. In the case of the Cream + (198 English + (8 + German) F₄) strain the number of mice is perhaps too small to permit much consideration. The Carter strain is in its origin related to the English; the tumor rate in this case stands on the border between a medium and high rate. The other strains in this class are hybrids in which either English, Carter, or Waltzer mice enter. In these hybrids there seems to be a tendency to an early tumor age. This would agree with the early tumor age of the English and Carter parent strains. In regard to the pure Waltzers, we have no statistics as to their tumor age and tumor rate. To judge from the result in the crosses we can only infer that their tumor age was early.

8. In a number of tumor strains with medium tumor rate the tumor age corresponds to the rate. (11 to 15 per cent of tumor mice in the I age period, an average of 45 to 55 per cent in the III age period.) In this group we include: European + No. 8, Heitler, German, 8 + German, No. 8, White Cream + I daughter of No. 10, Black Cream + European, Vermont Wild + English F₂ and German + No. 6 F₄. Of these, Black Cream + European and Vermont Wild + English stand on the border between a medium and late tumor age.

9. There are some strains with a medium tumor rate and a late tumor age. To this group belong: 101 (English) + (Euro-

pean + 103), European + English Tan, $8\frac{1}{2}$ + II daughter of No. 10, ($8\frac{1}{2}$ + II daughter of No. 10) + II d. of No. 10, Cream + I daughter of No. 10, and European + Cream. We notice that the parents (European + 103), European $8\frac{1}{2}$, and Cream have the tendency to impart to hybrid strains a late tumor age. All those parent strains have either themselves a late tumor age or their tumor age is on the border between medium and late, with exception of the European which, notwithstanding their relatively early tumors, likewise seem to impart a late tumor age to their hybrid offspring. In this strain there was perhaps present a greater individual variability in the tendency towards a certain tumor age. We notice furthermore that whenever the I or II daughter of No. 10 enters into a cross with a second parent strain with a tumor age which is late or approaches lateness, the No. 10 component of the cross, despite its high tumor rate, does not tend to make the tumor age earlier, because the European + I and II daughter of the No. 10 mice themselves have a relatively late tumor age considering their high tumor rate.

10. If we compare the list of the high tumor rate strains with that of the medium tumor rate strains, we find in the former the tumor age on the whole much more homogeneous than in the latter. In the high tumor rate strains the tumor age in all the important strains with exception of strains into which the daughters of No. 10 enter is early, while in the medium tumor rate strains the variability as to tumor age is much greater, although the deviations are about equally distributed on both sides of a medium tumor age, which latter represents the average.

LOW STRAIN TUMOR MICE

11. The Cream strain is typical of the low rate tumor strains. The difference in the tumor age between this strain and a typical high rate strain is striking; the tumors appear here much later in life than in the high tumor rate strain. We must of course take into consideration the fact that the smaller the number of tumor mice, the greater is the effect on the percentage age distribution of a small variation in the number of mice dying in the different age periods. Therefore we should expect a

Low rate tumor mice

STRAIN	NUM- BER OF MICE	TUMOR RATE	PERCENTAGE OF TUMORS IN			
			I age period	II age period	III age period	
			per cent	per cent	per cent	per cent
Total English Cream low rate hybrids.....	318	5.7	28.0 (2.5)	38.0 (3.4)	34.0 (3.0)	
(344 + Black Cream) F ₁ + Cream (white).....	11	0.0	0.0	0.0	0.0	
(English 344 + Cream, new individuals).....	143	9.0	23.4	37.0	39.6	
(English Sable 4444 + Cream)	175	2.8	30.0	36.0	34.0	
Total Cream.....	878	5.9	8.0 (1.0)	26.0 (3.3)	66.0 (8.2)	
(Total Cream ×).....	260	2.7	18.2 (0.8)	52.3 (2.3)	29.5 (1.3)	
(Cream × A).....	135	4.0	27.0 (1.4)	73.0 (3.8)	0.0 (0.0)	
(Cream × B).....	77	2.6	0.0 (0.0)	33.0 (2.0)	67.0 (4.0)	
(Cream × C).....	48	0.0	0.0	0.0	0.0	
(Old Cream).....	226	2.0	0.0 (0.0)	20.0 (1.2)	80.0 (4.3)	
(Cream A, mostly black).....	114	8.0	10.4 (1.7)	23.0 (3.7)	67.0 (11.0)	
(Cream B, mostly white color)	89	19.0	3.0 (1.1)	39.0 (14.0)	58.0 (21.0)	
(Among Cream B, June 1914 Family).....	40	35.0	4.6 (2.5)	33.5 (18.0)	61.9 (33.0)	
(The rest of Cream B after deduction of June 1914 family).....	49	6.0				
(Cream Black II).....	77	4.0	13.0 (1.3)	0.0 (0.0)	87.0 (8.5)	
(Cream Black III).....	98	11.0	13.6 (3.0)	14.0 (3.1)	72.4 (16.0)	
(Cream Y).....	14	0.0	0.0	0.0	0.0	
(Total new Cream).....	349	10.5	7.6 (1.7)	25.0 (5.6)	67.4 (15.0)	
Total European.....	263	9.0	21.0 (3.4)	34.0 (5.4)	45.0 (7.4)	
(European A).....	113	16.0	21.0 (6.0)	42.0 (12.0)	37.0 (11.0)	
(European B).....	150	3.3	18.0 (1.3)	14.0 (1.0)	68.0 (5.0)	
Total European + 102 or 103..	221	15.0	3.0 (1.0)	24.0 (7.5)	73.0 (23.0)	
(European + 102 or 103 A)....	146	20.5	4.3 (1.5)	23.0 (8.0)	71.7 (25.0)	
(European + 102 or 103 B)....	75	5.3	0.0 (0.0)	30.5 (5.5)	69.5 (12.5)	
(London 481).....	43	0.0	0.0	0.0	0.0	
Total London + (European + 103) F ₁	148	7.0	6.0	25.0	69.0	
[London + (European + 103) F ₁ A].....	85	5.0	13.0 (1.2)	30.0 (2.8)	57.0 (5.4)	
[London + (European + 103) F ₁ B].....	63	8.0	0.0 (0.0)	31.0 (4.1)	69.0 (9.0)	
415 = [101 English + (European + 103)]	80	12.5	12.0 (3.5)	41.0 (12.0)	47.0 (3.5)	
Total No. 8½.....	241	13.0	11.0 (2.9)	50.0 (16.0)	39.0 (10.0)	
(No. 8½ A).....	158	17.0	13.3 (4.0)	53.0 (16.0)	33.7 (10.0)	
(No. 8½ B).....	28	0.0	0.0	0.0	0.0	

STRAIN	NUM- BER OF MICE	TUMOR RATE	PERCENTAGE OF TUMORS IN		
			I age period	II age period	III age period
			per cent	per cent	per cent
(No. 8½ C).....	55	9.0	7.5 (2.0)	33.0 (9.0)	59.5 (16.0)
Total (European + 102) F ₁ + 8½ F ₄	602	16.0	6.5	21.0	72.5
[(European + 102) F ₁ + 8½ F ₄ A].....	473	16.0	7.0	18.0	75.0
[(European + 102) F ₁ + 8½ F ₄ B].....	129	15.0	8.6	28.5	62.9
Total (European + 103) F ₁ + IIIId of No. 10.....	219	14.6	4.4	29.0	66.6
[(European + 103) F ₁ + IIIId of No. 10 A].....	168	17.0	6.3	28.0	65.7
[(European + 103) F ₁ + IIIId of No. 10 B].....	30	13.0	0.0	10.7	89.3
[(European + 103) F ₁ + IIIId of No. 10 C].....	21	0.0	0.0	0.0	0.0
English Silver.....	127	7.0	35.0 (3.5)	65.0 (6.6)	0.0 (0.0)
English Silver Fawn.....	57	12.0	0.0 (0.0)	38.0 (11.0)	62.0 (18.0)
London + Silver.....	176	12.0	8.0	24.0	68.0
(No. 8 B).....	30	10.0	0.0	0.0	100
(8½ + IId of No. 10 B).....	28	14.0	0.0	100	0.0
German + Carter A.....	358	9.0	6.6	26.0	67.4
English Sable 344 + (European + English 146) (344-146)....	21	5.0	0.0	100	0.0
White Cream + (European 151 + Id of No. 10 = No. 697)	128	9.5	5.8 (1.65)	17.5 (5.0)	76.7 (22.0)
Cream + (Cream + European 428) F ₁	11	10.0	0.0	100	0.0
German + No. 8.....	112	0.0	0.0	0.0	0.0

much greater variability in the age distribution in the low rate than in high rate tumor strains. This does not, however, apply in the case of the representative strains of this group because we are working with a very great number of individuals in these strains. Thus, while in the Cream strain the tumor rate is as low as 5.9 per cent, the strain consists of 878 mice and among them are 52 tumor mice, a sufficiently large number for our purpose. If we consider, on the other hand, the substrains in this group, then the number of tumor mice usually becomes so small that the development of one or two more tumor mice in

an earlier age period may change considerably the age distribution. Notwithstanding this fact we find in the Cream strain that the smaller substrains behave essentially like the main strain; we notice a low percentage of tumor mice in the first age period and a high percentage in the third age period, with exception of the Cream X A substrain where we have to deal with only 5 tumor mice, two of which died in the first and three in the third age period, and even here a change in time of death in the case of one or two individuals would have made the age curve typical.

12. What applies in the case of the Cream strain applies also to the other more important low rate tumor strains, as for instance the European + 102 or 103 where, as a result of the somewhat higher tumor rate, we have to deal with 34 tumor mice. It also applies in the case of London + (European + 103) F_1 , in the large strain (European + 102) F_1 + $8\frac{1}{2}$ F_4 , where we can base our calculations on 97 tumor mice; furthermore, in the case of the (European + 103) + III daughter of No. 10, London + Silver, German + Carter A in which the tumor rate was only 9 per cent and the age distribution was accordingly typical for the low rate tumor strain, while in the German + Carter B, where the tumor rate rose, the percentage distribution in the different age periods also changed in the typical manner.

The same age distribution we find furthermore in the White Cream + (European 151 + I daughter of No. 10) and some smaller strains. In the German + 8 tumors did not develop. Wherever the European + 102 or 103 enter a cross, they not only depress the tumor rate, but call forth the typical age distribution characteristic of the low tumor rate strains. This applies even in the case of the English 101 + (European + 103) where the association with the English strain succeeded in raising the tumor rate to 34 per cent and thus in producing a medium rate tumor strain; but the tumor age is very nearly as late as that of the Cream.

13. There are a few groups in this class in which the tumors appear earlier, namely, European, a small substrain 415 = 101 English + (European + 103), No. $8\frac{1}{2}$, and English silver. Eng-

lish Silver is related to the English and the tumor age approaches that of the English. Notwithstanding the relatively small number of tumor mice in this strain, this age distribution is probably not altogether due to a coincidence; at least we may interpret in this sense the fact that even in the hybrids in which English Silver enters the tumor age is relatively early. In No. 8½ and in $415 = 101 + (\text{European} + 103)$ the tumor age stands at the border between the early and medium types, or rather it approaches the medium type. In the European strain the tumors appear earlier. Here we may have to deal with another instance in which tumor rate and tumor age are dissociated; in the case of the low rate tumor mice the number of tumor mice is, however, relatively so small that in individual strains, except the largest ones, chance variations cannot be excluded with certainty.

14. In the English-Cream hybrid with a low tumor rate the tumor age is very early, similar to that of the English. The number of mice is 318, the tumor rate is 5.7 per cent; we have to deal with 18 tumor mice. This corresponds to the similar high tumor age in the English-Cream hybrids with medium tumor rate. In this group the number of mice is still greater and our classification is based on the cancer age of 239 tumor mice.

We may therefore conclude that in the English-Cream hybrids, independently of the tumor rate which may vary in different cases, the tumor age approaches that of the English. This applies even in the case of the two individual English-Cream hybrids with a low tumor rate.

In the case of the English-Cream hybrids we find, therefore, a splitting in the inheritance of tumor rate and tumor age.

THE TUMOR AGE OF THE HIGH, AND LOW TUMOR RATE MEDIUM STRAINS

15. The great difference in the tumor age of the high, medium, and low tumor rate strains is brought out very clearly if we distribute all our strains among three great groups, according as to

whether their tumor rate is high, medium, or low, treating each group as a whole and determining in each of them the tumor age of the group.

Thus we obtain the following figures:

Total of the high tumor rate strains

I age period.	2741 mice	885 tumors = 32.3%	22.3%
II age period.	1176 mice	636 tumors = 54.1%	37.3%
III age period.	225 mice	132 tumors = 58.7%	40.4%
Average tumor rate 60.3%			

Total of the medium tumor rate strains

I age period.	4351 mice	394 tumors = 9.0%	14.6%
II age period.	2855 mice	635 tumors = 22.2%	36.0%
III age period.	1238 mice	374 tumors = 30.5%	49.4%
Average tumor rate 32.2%			

Total of the low tumor rate strains

I age period.	3971 mice	67 tumors = 1.69%	8.5%
II age period.	2747 mice	152 tumors = 5.53%	27.8%
III age period.	1362 mice	173 tumors = 12.7%	63.7%
Average tumor rate 9.9%			

This arrangement brings out very clearly the difference in the tumor age of the different groups. In the group composed of the high tumor rate strains the average tumor rate is 60.3 per cent, the tumors appear early, almost, but not quite, as early as in the English strain. The figures for the I, II, and III age period are I 22.3 per cent, II 37.3 per cent, III 40.4 per cent. In the second group the average tumor rate is 32.3 per cent. The tumor age is correspondingly intermediate. The figures for the 3 age periods are: I 14.6 per cent, II 36 per cent, III 49.4 per cent. In the third group the average tumor rate is 9.9 per cent. The average tumor age is approximately that of the Cream; the figures are I 8.5 per cent, II 27.8 per cent, III 63.7 per cent. We may therefore conclude that the higher the tumor rate in a strain, the earlier the tumors appear on the average; the greater is the number of tumors in the first age period, and the smaller is the number in the third age period. The differences in the first age period are, however, somewhat greater

than those in the third age period. The differences between the figures for the II age period, which represent the turning point are, as might be expected, much smaller in the three groups.

16. This relationship between tumor rate and tumor age can be shown in still another way. We may arrange all the strains in three different classes in accordance with their tumor age, the first class comprising those strains having a tumor age similar to that of the high tumor rate group, the second comprising those strains having a tumor age similar to that of the medium tumor rate group, and the third comprising the strains with a tumor age similar to that of the low tumor rate group.

We then find in the first class, comprising the strains with an early tumor age, 19 strains with a high tumor rate, 10 strains with a medium tumor rate, and 3 strains with a low tumor rate. Almost 60 per cent of the strains belong, therefore, to the high tumor rate strains.

In the second class, with a medium tumor age, we find 10 strains with a medium tumor rate, 2 strains with a low tumor rate, and 5 strains with a high tumor rate. In this class 59 per cent of the strains belong to the medium tumor rate strains.

In the third class, with a late tumor age, we find 14 strains with a low tumor rate, 6 strains with a medium tumor rate, and 1 strain with a high tumor rate. Sixty-seven per cent of the strains belong to the low tumor rate strains. In each class the majority of strains have a tumor rate which corresponds to the tumor age of that class.

ON THE PERIOD OF LIFE IN WHICH THE DEATH-RATE FROM CANCER IS HIGHEST IN MICE

17. It is generally assumed that in man the cancer incidence on the whole increases with increasing age. However, we find certain variations according to the kind of cancer which is considered; thus the death-rate from mammary and uterine cancer reaches a somewhat earlier maximum than the death-rate from cancer in general. The same applies to sarcoma. In mice Murray finds the death-rate from mammary cancer to reach a maximum at the age of thirteen to sixteen months. Murray's

statistics differ from our own in that they are based on a much smaller number of mice and in the method of computation he uses. Later we shall apply the same method also to our material.

We found that the age of death from mammary cancer in mice is not a fixed point, but varies with the cancer rate. The cancer rate being variable in different strains, the cancer age is likewise variable. In general we may state that the cancer age is the earlier, the higher the cancer incidence.

18. If we consider the total figures for high, medium, and low tumor rate strains respectively, we find that in the high tumor rate strains the difference between the cancer rates in the third and second age periods is very small, but the cancer rate is slightly higher in the third age period. In mice above the age of eighteen months the cancer rate is therefore slightly higher than in mice between the age of twelve and eighteen months. The maximum still is in the third age period.

In the medium tumor rate strains the difference between the cancer rate in the second and third age periods is more marked; the maximum is here quite definitely in the third age period. In the low tumor rate strains the difference between the tumor rate in the second and third age periods is still much greater; the maximum is very decidedly in the third age period. The lower the tumor rate, the more decidedly the maximum moves to the third age period. The lower the tumor incidence, the greater the maximum reached in the third age period and the greater the difference between the tumor incidence in the second and third age period. The difference between the tumor rates in the first and second age periods varies less; it happens to be least in the group of the high rate tumor strains.

19. If instead of considering the groups as a whole, we consider the individual strains composing them, the difference in the situation of the maximum tumor incidence in different strains comes out still more clearly. Thus in the $8\frac{1}{2} + 328$, a high tumor rate strain, the maximum is decidedly in the second age period, while in the English, although they have a slightly higher tumor rate, the maximum is somewhat higher than in the $8\frac{1}{2} + 328$, although it is still in the second age period if we consider the

total strain; the difference between the second and third age periods is however very small in this case and in the substrains we find some variation as to the maximum.

The great majority of the English hybrid strains behave like the English strain as far as the maximum of the tumor incidence is concerned. Similarly to the English strain behave in this respect particularly the English-Cream hybrids and European + English Tan, irrespective of their tumor rate.

In addition to various English hybrids, the strains London, 8 + German, and 8½ show a maximum similar to that of the English mice. On the other hand, in the European 151 + I or II daughter of No. 10, in which the tumor incidence is very high, the maximum is decidedly in the third age period.

We must then conclude that the maximum of tumor incidence is found at a different age level in different strains, and that this peculiarity is transmitted by heredity in the same way as the other characteristics of the strain.

The data as to the age period in which the maximum cancer incidence is found in mice, which we present here, permit only comparative conclusions. We are at present operating with three age periods which are not equal in length and we cannot therefore state at which age period the maximum occurs. But our data have a relative value. They show the shifting of the age period in different strains in dependence upon the variations in the cancer rate of the various strains and groups. And we may furthermore conclude that in all statistics so far no account has been taken of this variability of the tumor age in general and of the maximum in the tumor rate in particular. All available statistics give an average, in which this variable factor is disregarded and in which necessarily the result will differ in accordance with the relative preponderance of high or low tumor rate families or strains.

20. If instead of considering the three groups of mice separately we determine the tumor incidence in the three age periods in all the mice, irrespective of the group to which they belong, we obtain the following figures: In the first age period there were 11,063 mice; 1346 mice developed tumors during this period = 12.2 per cent.

In the second age period there were 6778 mice with 1423 tumor mice = 21 per cent.

In the third age period there were altogether 2825 mice, with 679 tumor mice = 24 per cent.

If we calculate these figures on the basis of a tumor incidence of 100 per cent, we find for the first age period a tumor incidence of 21 per cent, for the second age period an incidence of 36.7 per cent, and for the third age period an incidence of 42.3 per cent. These figures are somewhat between those of the high and medium tumor rate mice, but they approach very closely those of the high tumor rate mice. The maximum of the tumor incidence is accordingly in the third age class, but the difference between the figures for the second and third age period is relatively small.

21. If instead of considering in each age period the number of mice alive at the beginning of this period and the number of mice developing tumors in this period, and on this basis determining the percentage figures of tumor incidence for each age period, we determine what percentage of mice will develop cancer during the whole period following this date irrespective of the number of mice eliminated through death during each period, we obtain a different result. We had altogether 11063 mice which reached the age above six months. Of these 3448 subsequently developed cancer = 31.2 per cent; of 6778 mice which reached the age of twelve months, 2102 later developed cancer = 31 per cent; and of 2825 mice reaching the age of eighteen months, 679 developed cancer = 24 per cent. Figured out on this basis the tumor rate is similar for mice reaching the age of six and of twelve months. In these groups the tumor rate is higher than in mice reaching the age of eighteen months.

This mode of computation seems to us less satisfactory than the first method used, because it includes the number of individuals dying in each period among those which are liable to be affected by cancer in subsequent periods, and it thus makes the tumor incidence in the first two age periods too high.

22. If we leave out of consideration the mice dying below the age of 6 months we find among our material altogether a tumor incidence of 31.2 per cent. This is a relatively high tumor rate

which presumably exceeds the average tumor rate among white mice in unselected strains. Our material is a selected one, in which a special value was attached to high tumor rate strains.

23. If we determine with the second method the cancer incidence in the various groups of high, medium, and low tumor rate mice, we find the following figures.

High tumor rate mice

I	2741 mice	1653 tumor mice = 60.3%
II	1176 mice	768 tumor mice = 65.3%
III	225 mice	132 tumor mice = 58.7%

Medium tumor rate mice

I	4351 mice	1403 tumor mice = 32.3%
II	2855 mice	1009 tumor mice = 35.3%
III	1238 mice	374 tumor mice = 30.2%

Low tumor rate mice

I	3971 mice	392 tumor mice = 9.9%
II	2747 mice	325 tumor mice = 11.7%
III	1362 mice	173 tumor mice = 12.7%

On this basis the percentage figures for the different age periods are very similar in the three groups. But in the high and medium tumor rate mice the maximum of the tumor incidence is in the second age period, in the low tumor rate mice it is again in the third age period. Even this method, which is more faulty than the first one, brings out the shifting in the tumor incidence with varying tumor rates.

THE INHERITANCE OF TUMOR AGE AND INCIDENCE IN HYBRIDS

24. We have already referred to the behavior of individual hybrid strains as far as the relation between tumor age and tumor rate is concerned. We have also referred to some of the larger groups of hybrids. We shall now discuss the behavior of hybrids in some of the larger groups connectedly and compare their tumor rate and age with that of the parent strains.

(a). *English-Cream hybrids*. The figures for the parents are as follows:

A. Total English

I	689 mice	280 tumor mice = 40.6%	25%	67.6%
II	258 mice	161 tumor mice = 62.5%	39%	
III	46 mice	25 tumor mice = 54.5%	36%	

B. Total Cream

I	878 mice	9 tumor mice = 1.0%	8%	5.9%
II	600 mice	20 tumor mice = 3.3%	26%	
III	279 mice	23 tumor mice = 8.2%	66%	

C. Total English-Cream Hybrids

I	1057 mice	92 tumor mice = 8.6%	20%	24.3%
II	668 mice	120 tumor mice = 18.0%	42%	
III	280 mice	45 tumor mice = 16.1%	38%	

In these hybrids the tumor rate is on the whole intermediate between those of the parents, although somewhat nearer the Cream parent than the English. The tumor age, on the other hand, is almost that of the English parent. In this case we have to deal with such large figures that we are justified in assuming that these figures express causal relations and are not due to accidental findings.

The same relation comes out still more clearly if we divide the English-Cream hybrids into three subgroups.

A. Total English-Cream Hybrids with a medium tumor rate

(and with a slight admixture of high rate tumor mice)

I	434 mice	53 tumor mice = 12.0%	20%	32%
II	264 mice	67 tumor mice = 25.0%	41%	
III	100 mice	21 tumor mice = 23.0%	39%	

B. Total English-Cream Hybrids with a low tumor rate

I	318 mice	8 tumor mice = 2.5%	28%	5.7%
II	205 mice	7 tumor mice = 3.4%	38%	
III	101 mice	3 tumor mice = 3.0%	34%	

In this case the tumor rate is altogether that of the Cream, but again the tumor age is that of the English parent strain.

C. Total English Tan (121) + Cream

I	267 mice	32 tumor mice = 12%	22%	31%
II	173 mice	36 tumor mice = 21%	38%	
III	63 mice	14 tumor mice = 22%	40%	

Also in this strain of hybrids the tumor rate is intermediate while the tumor age is that of the English parent strain.

b. The same conclusion, that factors for tumor rate and age may be transmitted independently of one another, follows from a consideration of the hybrids between Cream and European 151 + I daughter of No. 10.

The tumor rate and tumor age of the Cream we have already given.

Figures for the mother strain European 151 + I daughter of No. 10 are as follows:

I	254 mice	63 tumor mice = 25.0%	15.0%	
II	148 mice	120 tumor mice = 81.0%	33.0%	72.0%
III	46 mice	39 tumor mice = 88.0%	52.0%	

*The Total Hybrids Cream + (European + I daughter of No. 10)
(three different crosses used)*

I	498 mice	22 tumor mice = 4.4%	8.4%	
II	365 mice	53 tumor mice = 15.0%	29.0%	27.5%
III	189 mice	62 tumor mice = 33.0%	62.6%	

In this case the tumor rate is again on the whole intermediate and slightly nearer that of the Cream, but the tumor age is that of the Cream, contrary to what we found in the former hybrids. If we omit from the table of hybrids one cross in which the tumor rate was low, the figures for the Cream + 10 hybrids with medium tumor rate (2 crosses) are as follows:

Total Cream + (European + I daughter of No. 10) hybrids with medium tumor rate

I	370 mice	20 tumor mice = 5.4%	10%	
II	286 mice	49 tumor mice = 17.0%	32%	34%
III	161 mice	56 tumor mice = 31.0%	58%	

Again the tumor rate is intermediate and the tumor age is almost that of the Cream. In this connection it is of interest to remember that the tumor age of the European 151 + I or II daughter of No. 10 is later than that of the English, and accordingly it has a tendency to transmit to crosses a later tumor age than the English.

If we cross strains in which tumor rate and tumor age differ less than in the preceding strains, we again obtain a tumor rate

and tumor age intermediate on the whole, or perhaps resembling slightly more one parent than the other. This is shown in the following crosses:

A. German

I	42 mice	8 tumor mice = 19%	15.5%	
II	19 mice	7 tumor mice = 37%	30.3%	40.5%
III	3 mice	2 tumor mice = 66%	54.2%	

In this case the number of mice is rather small; we can therefore use it only with caution.

B. Total No. 8

I	243 mice	17 tumor mice = 7%	12.0%	
II	150 mice	24 tumor mice = 16%	27.4%	27.5%
III	78 mice	26 tumor mice = 35%	60.6%	

C. Total No. 8 + German

I	373 mice	30 tumor mice = 8%	14.0%	
II	280 mice	66 tumor mice = 24%	42.0%	34.0%
III	123 mice	31 tumor mice = 25%	44.0%	

The tumor rate of the hybrids is about intermediate and the tumor age is very similar in both parent strains as well as in the hybrids.

d. Another strain may be of interest; this resembles in its character the last named hybrid strain and is related to it. We crossed the 8 + German with an English male, the offspring of Tumor mouse 198.

The data for the parent strains have already been given. The figures for the cross are as follows:

English (198) + (8 + German)

I	217 mice	61 tumor mice = 28%	20%	
II	89 mice	52 tumor mice = 58%	41%	54%
III	9 mice	5 tumor mice = 55%	39%	

In this case the tumor rate of the hybrids is again intermediate, but slightly nearer that of the English. The tumor age is also intermediate, but again somewhat nearer that of the English. In this case no distinct split between tumor rate and tumor age occurred in the hybrids.

25. We believe that in these investigations we have established a definite relation between tumor incidence and tumor age. In general the average tumor age is the earliest in those strains of mice in which the number of individuals affected by cancer of the breast is greatest. There is in addition a difference in the tumor age in a number of strains in which the tumor rate is similar; or, conversely, in a number of strains in which the tumor age is similar the tumor rate may differ. We have seen furthermore that in crosses tumor age and tumor rate may be inherited independently of each other, or in other cases the tumor age may correspond to the tumor rate. How can these facts be interpreted in accordance with the current theories of heredity? We may assume as most probable that the tendency to cancer depends on the presence of multiple factors.

A certain number of these factors must be present in an individual if a tumor is to appear. These factors determine the intensity of the tendency towards cancer in each individual; but this intensity tends not only to cause a tumor to appear in a certain animal, but it has the tendency to make it appear at an early period of life. Tumors appear the earlier, therefore, the greater the number of tumor factors present or the greater the importance of the factors represented. We may assume that different strains differ greatly in the number and character of these factors present in the average of the individuals. In the English and $8\frac{1}{2} + 328$ there may be in an individual affected with cancer, on the average, a greater number of the more effective factors than in a Cream individual which happens to have just a sufficient minimum of factors to insure the appearance of a tumor. Therefore the intensity in the tendency towards the appearance of tumors is greater in the English and $8\frac{1}{2} + 328$ than in the Cream. In all these individuals tumors appear, but the greater intensity in the English and $8\frac{1}{2} + 328$ causes the tumors to appear earlier in life in these latter strains. There are, of course, variations in the individuals of different strains, and some individuals of the $8\frac{1}{2} + 328$ or English may resemble the average Cream as far as the character and number of their tumor factors are concerned. There may be, in addition, among the

multiple factors determining tumor growth, some special factors which determine more directly the tumor age, and these special factors may be inherited independently of the multiple factors which determine mainly the appearance of a tumor.

These special factors may overcome the effect of the ordinary tumor factors, which in themselves already have a tendency to affect the tumor age. If, on the other hand, we assume that factors determining tumor age are always distinct from those determining tumor incidence, then we must conclude that both sets of factors are usually linked to each other in such a way that, in general, in those strains in which the tumor rate is high the factors determining an early appearance of tumors are also present; but that it is possible to dissociate these two sets of factors in certain cases, especially through hybridizations. Both interpretations are related to each other; the first interpretation including the second one. These interpretations must at present, of course, be considered as merely of tentative character, but they seem best to represent the facts so far established.

CONCLUSIONS

1. The tumor age of a certain strain is as definitely determined by heredity as the tumor incidence; the tumor age may be a finer means of distinction between different strains than the tumor incidence.

2. There is a definite relation between tumor rate and tumor age in mice. In those groups or strains in which the tumor incidence is great the tumors tend to appear early, and in those groups or strains in which the tumor rate is low the tumors tend to appear late. We can arrange our mice in three groups, with high, medium, and low tumor rate; in these three groups the tumor age shows corresponding changes.

3. The period of life at which a certain kind of tumor shows a maximum frequency in a certain species is not definitely fixed, but it varies with the rate of tumors in certain strains. The usual statistics represent an average between the maxima in different strains in which the maximum varies in accordance with their tumor rate.

4. In addition to this general relation between tumor age and tumor rate, there is in certain strains a specific tumor age which may differ from that expected in the strain on the basis of its tumor rate. Furthermore, in hybrid strains tumor rate and tumor age may be inherited independently of each other.

5. These relations between tumor age and tumor rate can best be explained if we assume that the hereditarily transmitted constitution, so far as it represents the tendency of the organism to develop tumors, depends on the coöperation of multiple factors. These multiple factors determine the intensity in the tendency to tumor development in a certain individual. In general, the greater is this intensity, the earlier do the tumors appear and the greater is the probability that in related individuals there exists likewise a tendency to the development of tumors.

It is furthermore probable that in addition to the general factors determining the intensity in the tendency towards the development of cancer, there exist factors which determine specifically the tumor age in certain individuals and strains.

REFERENCES

- (1) LATHROP, A. E. C., AND LOEB, LEO: *Proc. Soc. Exper. Biol. and Med.*, 1913, xi, 34; *Jour. Exper. M.*, 1915, xxii, 646 and 713, 1918, xxviii, 475; *Jour. Cancer Res.*, 1919, iv, 137.

FURTHER INVESTIGATIONS OF DISTURBANCES OF BLOOD SUGAR EQUILIBRIUM IN THEIR RELATION TO NEOPLASIA¹

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In previous publications (1), it has been shown that the injection of a protein into the animal body is followed by disturbance of the blood sugar equilibrium, and that this disturbance of sugar equilibrium can be influenced (either increased or decreased) by the ablation of one or more of the endocrines (2). The present report concerns a further study of: (a) the interrelationship of the endocrines in the regulation of blood sugar equilibrium; (b) the relation of the degree and type of blood sugar disturbance after the injection of protein to the strength of the anti-bodies developed; (c) the relation of the type and intensity of the reaction following the injection of homologous protein to neoplasia in man.

I. EXPERIMENTAL PART

The technical steps of the method employed are simple. The animal to be tested is starved for a period of twelve hours. Just before, and again sixty minutes after the subcutaneous injection of the antigen chosen, the blood sugar values are determined in milligrams per 100 cc. The lower of the two values is subtracted from the higher, the difference being taken to indicate the degree of disturbance as measured in milligrams. In the present work, in determining the blood sugar values

¹ We beg to express our appreciation to all of the attending physicians and surgeons of the Lenox Hill Hospital for the clinical material contained in this report.

we have used either the method of Epstein or of Kliner, or the Wallace and Gallagher modification of the Folin-Wu method. We have modified the Wallace and Gallagher method in that the blood is measured instead of weighed, 0.1 cc. being used. For this purpose a standardized pipet, so made that the capillary bore necessary to contain the specified amount is about 15 cm. long, was employed. It is essential that the same worker make all determinations in a given experiment and that the individual be skilled in the technical steps of the method chosen. The same method for determining the sugar values should be used throughout any one experiment. These conditions have been complied with in the work recorded here.

In our early experiments it was found that in normal animals after the injection of a given antigen, different types and degrees of reaction occurred. For example, one animal might show an increase of 100 mgm. in the blood sugar, while a second treated exactly as was the first might show an increase of but 15 mgm. A third animal, on the other hand, instead of showing an increase might show a decrease, for example, of 25 mgm. Hereafter, we shall use the terms "plus type" and "minus type" to designate, respectively, reactions in which the sugar concentration rises, and reactions in which a decrease occurs.

In investigating these variations the first factor considered was the influence of sex and age upon the type of reaction. A number of young female, young male, old female, and old male rats were tested in accordance with the method previously outlined, the injected antigen being 0.5 cc. of a 1 per cent heterologous protein (beef peptone). The averaged results of this experiment show that in the old males the plus and minus reactions occurred with the same degree of frequency, while in young and old females and in young males the plus reactions slightly predominated.

In a second experiment, one or another of the endocrine glands was removed, the test being performed at varying intervals after ablation. A 1 per cent heterologous protein (beef peptone), in doses of 0.5 cc., was first used as antigen, and after an interval of several days a similar dose of a similar strength

of homologous protein (rat spleen) was employed. The glands removed were as follows: both testes complete, both ovaries complete, spleen complete, thymus complete, thyroid and embedded parathyroids about 95 per cent entire; both adrenals complete; pancreas about 90 per cent entire. As controls, normal animals and animals from which one kidney had been removed were used. There were from 6 to 12 animals in each group.

The data of the experiments are given in table 1. The percentages of plus reactions are about the same in the normal controls, in those with one kidney removed, and in the thymus-

TABLE 1

ORGAN REMOVED	PERCENTAGE GIVING PLUS REACTIONS		PERCENTAGE GIVING MINUS REACTIONS	
	Homologous protein	Heterologous protein	Homologous protein	Heterologous protein
Normal controls.....	70	65	30	35
Kidney.....	60	55	40	45
Thyroid.....	0	100	100	0
Thymus.....	39	20	66	80
Spleen.....	83	16	16	83
Adrenals.....	100	100	0	0
Pancreas.....	58	67	42	33
Testes.....	0	0	100	100
Ovaries.....	78	75	22	25

free, pancreas-free, and ovary-free groups, irrespective of which antigen was used. There was a difference in the types observed in the spleen-free group, for of these 16 per cent gave a plus reaction when injected with heterologous protein, as compared with 83 per cent giving a plus reaction when homologous protein was used as antigen. One type of reaction only was observed in three groups: the adrenal-free and thyroid-free groups gave 100 per cent plus reactions, and the testes-free 100 per cent minus reactions when injected with heterologous protein. When homologous protein was used as antigen the adrenal-free and testes-free groups reacted in the same manner, while the thyroid-free groups gave 100 per cent minus instead of plus reactions.

Having observed the reactions after single gland removal, and in that manner having obtained groups in which all the animals reacted alike as regards type, we studied, in a third experiment, the effect of temporary overactivity of various glands. Extracts, in the proportion of 5 grams of the dried powdered gland in 25 cc. of physiological saline solution, were used as antigen, the dose being 0.5 cc. In this fashion extracts of the pituitary, pancreas, adrenal, and thyroid glands of sheep were prepared, and because these extracts, aside from their supposed specific secretion content, were also heterologous protein, a similar extract of dried sheep liver was used, as a control. The other details of the experiment were similar to those previously described. There were 12 non-operated rats in each group. The data of this experiment are given in table 2.

TABLE 2

GLAND EXTRACT INJECTED	PERCENTAGE PLUS REACTIONS	PERCENTAGE MINUS REACTIONS
Liver.....	58	42
Pituitary.....	50	50
Pancreas.....	25	75
Adrenal.....	42	58
Thyroid.....	67	33

In the animals injected with heterologous protein derived from the adrenal and thyroid glands there was little or no change in the percentages of the two reaction types as compared with the group injected with heterologous liver protein. When pancreas extract was injected, however, there was a predominance of the minus reactions, which is in contrast to the pancreas-free animals in which injections of heterologous protein produced a predominance of plus reactions.

The fact that, after single gland removal, but one type of reaction occurred in certain groups led to a study of the relationship of the endocrine chain in its effect upon the reaction types. Double gland ablations were done in such fashion that all possible two gland combinations were obtained, always excepting those with the pituitary.

Six rats were used in each group. At varying periods after operation these animals were injected with 0.5 cc. of homologous protein and sugar estimations were made as before. The data of the experiment are given in table 3.

When compared with single gland removal, double gland ablation showed the following changes in type of reaction. The spleen and testes combination showed results opposite to those of spleen and ovaries, plus types predominating with the former and minus types with the latter. The thymus-thyroid combination gave a predominance of plus types while the thymus-adrenal gave a majority of minus reactions. Three groups showed one type of reaction throughout; the adrenal-pancreas, testes-pan-

TABLE 3

	TESTES		OVARIES		SPLEEN		THYMUS		PANCREAS		ADRENAL	
	+	-	+	-	+	-	+	-	+	-	+	-
Thyroid.....	67	33	0	100	50	50	67	33	80	20	40	60
Adrenal.....	50	50	80	20	50	50	32	68	0	100		
Pancreas.....	0	100	25	75	60	40	52	48				
Thymus.....	60	40	67	33	60	40						
Spleen.....	75	25	25	75								

+ = plus reaction percentage

- = minus reaction percentage

creas, and the thyroid-ovary combination gave 100 per cent minus reactions.

As has been stated in a previous paragraph not only did normal animals differ in type of reaction, but they also differed in the intensity of the blood-sugar disturbances. If the intensity of the reaction, as measured by the difference in milligrams per 100 cc. of the two sugar estimations, be compared in the young and old (chart 1) it is found that the young react more distinctly than do the old. Of the two sexes the males react more distinctly than do the females.

If the intensity of the reaction which follows the injection of heterologous protein be compared with that which follows the injection of homologous protein in normal and in gland-free

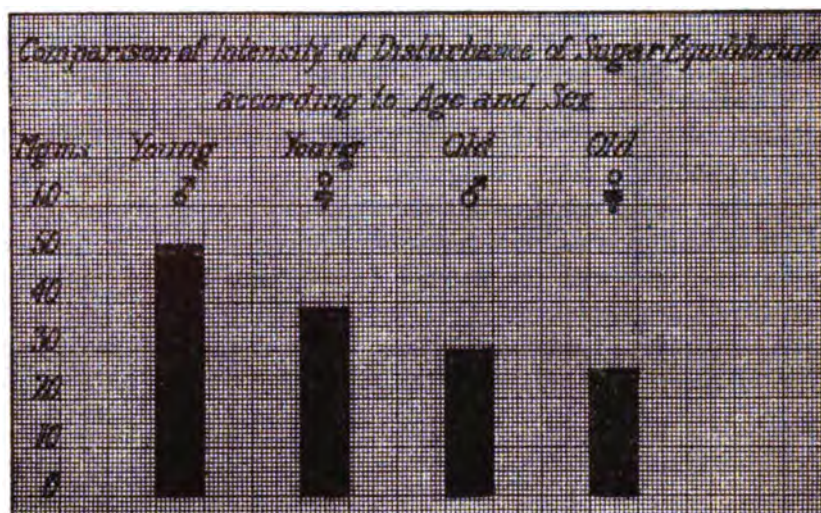
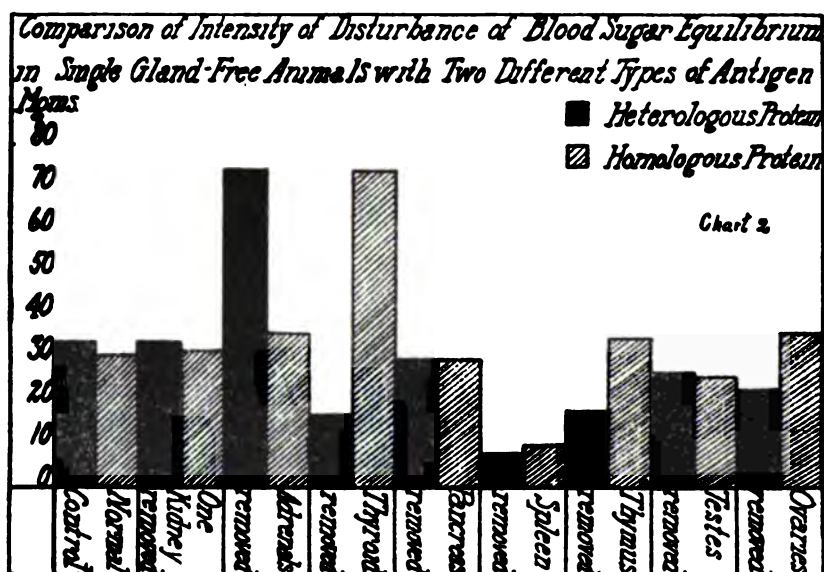


CHART 1



animals (chart 2), it is found that the same degree of reaction occurs with either antigen except in animals with the adrenals or the thyroid removed. The adrenal-free group reacted more distinctly to heterologous protein, while the thyroid-free reacted more intensely to homologous protein. Spleen-free animals showed a lessened intensity of reaction to both antigens.

When the reaction following injection of homologous protein is studied in animals from which two glands have been removed, the results (2), to quote from the paper previously referred to, are as follows: "There are two combinations of endocrines which when ablated inhibit the reaction; these are (1) spleen and either gonad set, and (2) adrenal and pancreas. If one gland from either system be ablated and with it one gland from the other system, there results little or no change from the normal reaction. If, however, one gland from one system be removed and with it one gland from another system, and in addition either the thyroid or thymus, then marked inhibition occurs. Removal of one gland from one system and either thyroid or thymus does not produce inhibition. If both glands be removed from each system, then, in order to induce inhibition, both of the intermediate glands, i.e., thyroid and thymus, must also be removed; the ablation of one intermediate gland is not sufficient. From this it appears that there are two systems which control the reaction and these systems are connected through two apparently interchangeable glands."

Stimulation of the reaction with double gland ablations occurred in two systems, analogous to those producing inhibition. These systems were the thyroid in combination with either ovaries, pancreas, or spleen, and the pancreas in combination with either testes, thyroid, or spleen.

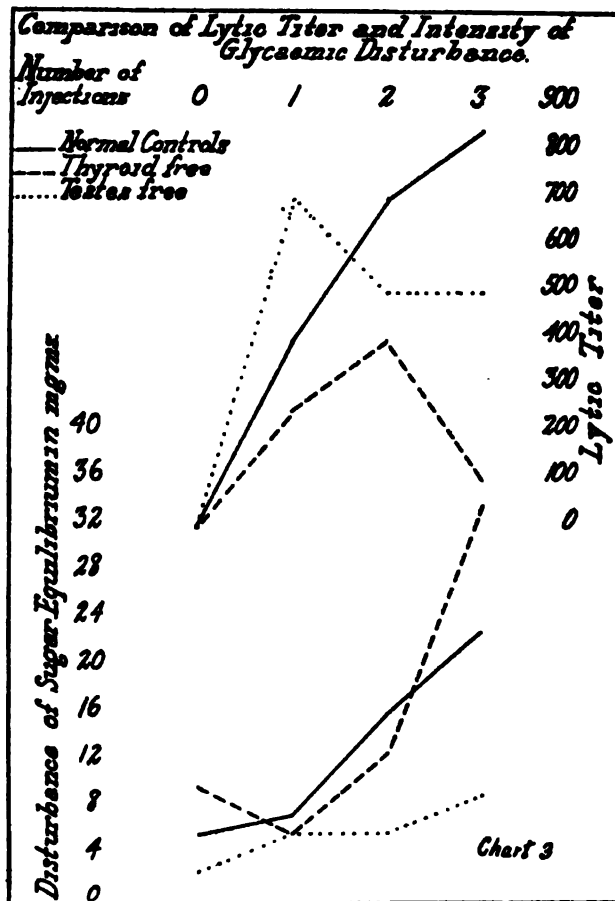
The exact significance of variations in type and in intensity of reaction is somewhat in doubt. It might be supposed, since disturbances of blood-sugar equilibrium follow the injection of an antigen until the injected animal has exhausted the power to produce further antibodies against the antigen injected, that variations in the type or in the intensity might be indicative of the relative strength of the antibodies formed.

Whether such an hypothesis is valid or not was determined in the following experiment. Rabbits, in groups of four animals each, were prepared as follows: one group served as normal controls; from a second the testes were removed; while from a third the complete thyroid apparatus was excised. One week after operation and after twelve hours' starvation blood-sugar estimations were made, the animal was then injected intraperitoneally with 2 cc. of a 50 per cent suspension of washed sheep red cells, and one hour after the injection the blood sugar was again determined. Lytic titers were made with serum obtained from the first bleeding. Two subsequent injections and double sugar determinations as well as lysin titers were made at weekly intervals, and a final lytic titer was made one week after the last injection. As is shown in chart 3 the thyroid-free animals showed a greater sugar disturbance than did the control animals, and the control animals in time showed a greater disturbance than did the testes-free animals. If intensity of reaction bears relation to the strength of antibody formation then we should expect the thyroid-free group to show the highest lytic titer, and the testes-free the lowest titer. Such, however, was not the case, for the normal controls gave the highest titer, the testes-free next, and the thyroid-free the lowest titer.

There being no demonstrable relationship between the intensity of the reaction and the strength of the antibody development, the most plausible remaining explanation is that the phenomenon is an indication of the relative activity of various of the endocrines. The failure of the injected animal to react after the maximum antibody production has been attained might also be taken as an indication of the temporary exhaustion of the endocrines.

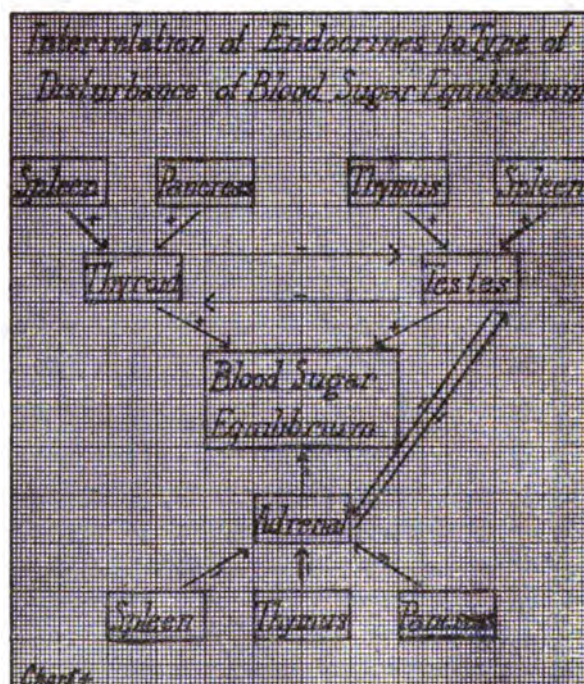
It is more logical, in endocrine studies, to consider averaged group behavior rather than individual behavior, and it is on averaged group behavior that the scheme of interrelationship given below has been worked out. If the ablation of a gland or of a group of glands is followed by a minus reaction, then that gland or the combination when in situ may be considered as causing a plus reaction. Similarly, if a given gland or gland

group removal brings about an inhibition, then that particular combination when in situ could be considered as stimulators and vice versa. The deviation one way or another from the behavior of the control group of normals would serve in lesser degree to indicate further interglandular relationships.



Thus as is diagrammatically shown in chart 4, if the injection of a homologous protein is followed by a drop in the blood-sugar concentration, either the thyroid or, if the individual be a male, the testes, may be considered as inefficient; whereas, if the sugar values rise, the adrenals may be considered as overactive. It

may also be supposed that decreases in the sugar concentration are due to lessened activity of the adrenal whose function is to keep up the sugar concentration. This insufficiency may be due either primarily to the adrenal or secondarily to the ineffectiveness of the stimuli reaching that gland through the spleen, thymus, or pancreas. In a similar manner, a rise in the blood-sugar concentration may be attributed to failure of the thyroid or testes to hold the blood sugar at the lower level, and this



may in turn be due to the thyroid or testes primarily, or it may occur secondarily because of failure of the stimuli from either spleen, pancreas, or thymus.

The intensity of the reaction has been worked out on similar lines. Accepting, for example, that a normal intensity of reaction for the rat is 31 mgm., if a gland removal results in reaction intensity of but 10 mgm. then the gland removed may be considered when in situ to be one of those helping to keep up

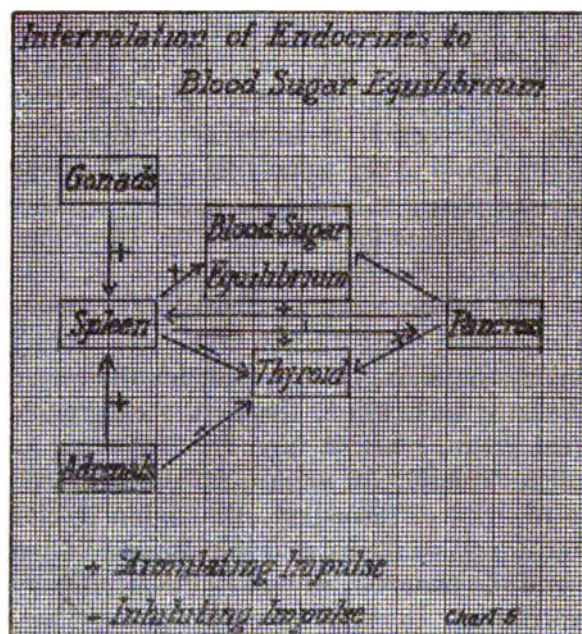
the normal intensity. If two glands be removed from two different groups and it is found that group A has a reaction intensity of 45 mgm. and group B a reaction intensity of 10 mgm. it may be accepted that the gland whose removal brought about the increased intensity, is when in situ one of the group which keeps the intensity down, the opposite being the case in the group in which gland removal caused a less intense reaction. Now, if both glands be removed from a third group and the reaction intensity of this group is found to be 10 mgm., it may be supposed, gland A in situ being an inhibitor, and gland B in situ being a stimulator, that gland A sends an inhibiting influence to gland B, for gland A being removed permits gland B to act without restraint, and the reaction is intensified. Gland B being removed permits gland A or some other gland to act without restraint in consequence of which the reaction intensity falls.

The intensity of reaction according to glandular interrelationship is indicated in chart 5. For example, if the adrenal be removed, the spleen lacks a stimulating influence and the thyroid an inhibiting one; the retention of a normal reaction intensity might, therefore, be expected, for the plus and minus influences balance each other, the actual experimental values being: normal group, reaction intensity 31 mgm.; adrenal-free group, reaction intensity 36 mgm. Ablation of the spleen results in the removal of secondary stimulation received via the gonads, adrenal, and pancreas, and the removal of a stimulating impulse sent to the pancreas, as well as directly upon the blood-sugar concentration. It might be expected, therefore, that the splenic removal would depress the intensity of the reaction. The actual experimental values obtained were: normal animals, reaction intensity 31 mgm.; spleen-free group, reaction intensity 10 mgm.

Theoretically, therefore, if the blood sugar concentration after an injection of homologous protein falls distinctly, it may be supposed that the mechanism which causes increased concentration (chart 4) has not responded or that the mechanism which depresses has been stimulated. An intense reaction would speak for thyroid inhibition. In contrast, if the reaction

were slight, say 10 mgm., splenic inhibition would be suspected. Inversely, if the sugar concentration rises distinctly it would indicate adrenal hyperactivity in combination with thyroid and pancreas, while if the reaction were minimal it would indicate adrenal hyperactivity in combination with spleen and gonad.

That disturbances of blood-sugar equilibrium no longer occur after the injection of protein, when the organism has attained



its maximum antibody producing power is, on this basis, to be interpreted as indicating temporary exhaustion of certain of the endocrines.

CLINICAL APPLICATION

It has already been noted that a majority of the mice bearing spontaneous tumors subjected to this test have shown a decreased intensity of reaction. In order to follow this lead, further investigations were carried out on human beings suf-

fering from a large variety of pathological conditions. The technic of the test as applied to the human cases was as follows:

After the evening meal no medication was given, the patient was starved until the last specimen of blood was withdrawn the following morning. Just before and again 45 and 120 minutes after the subcutaneous injection of 10 c.c. of homologous protein, blood was withdrawn and the sugar content determined. The degree of disturbance of equilibrium was estimated by subtracting the lowest from the highest of the three values obtained, the difference in milligrams being the standard of comparison. The homologous protein used was either blood serum or ascitic fluid obtained from individuals free from syphilis, tuberculosis, or cancer, in order to obviate any possible chance of disease transmission. The sterility of the fluid was assured by culture. No reactions were encountered as a result of such injections except in one instance where there was a slight chill followed by a rise in temperature to 102°F. With the Folin-Wu method we have found that not more than three unknowns can be compared with the same standard because the colors of the standard fade rapidly.

The clinical diagnoses have in every instance been confirmed either by histological or bacteriological examination, autopsy, operation, or investigation of the subsequent course of the patient. All the cases of neoplasia have been confirmed microscopically.

Before proceeding to an analysis of the data obtained as a result of these investigations it is profitable to revert to those obtained during investigations already reported (3). In this previous investigation blood-sugar equilibrium was disturbed by the oral administration of 100 grams of anhydrous glucose. When the individuals examined by this method were divided into male and female, cancerous and noncancerous, the following results were obtained. There was no predominance of type in one group over another, i.e., a particular type of reaction could not be said to be characteristic of any given group. If the intensity of the reaction, i.e., the degree to which blood-sugar equilibrium was disturbed was compared (chart 6) it was found

that with the exception of the non-cancerous males all the groups gave the greatest percentage of cases with a reaction intensity of from 61 to 120 mgm.; in brief, there was nothing characteristic of neoplasia.³

However, when the data of the types and intensities of reaction observed after the injection of homologous protein in man were similarly analyzed there were some very interesting and suggestive variations.

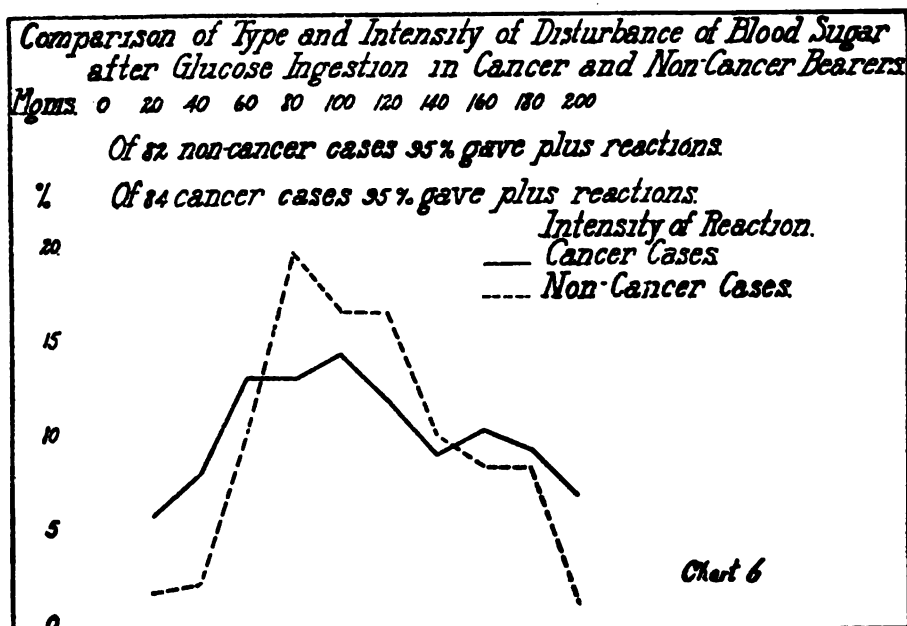


Table 4 gives the statistical data of the 209 cases which have been examined. When this information is presented as in table 5, several points are evident; a larger percentage of the

³ In a recent publication Killian and Kast (4) stated that the hyperglycemia observed in cases of neoplasia is attributable to the nitrogen retention present in many of these cases. This factor had not been considered by us in our previous communications. A review of our data, however, does not bear out the contention advanced by these authors, but confirms in a very emphatic manner the observation made by Spence (5) who attributed the hyperglycemia to the age of the individuals. In his investigations Spence showed that hyperglycemia occurs with increasing frequency in the more advanced age periods.

TABLE 4

CASE NUMBER	DIAGNOSIS	DISTURBANCE OF BLOOD SUGAR
		<i>mgm.</i>
6432	General carcinomatosis.....	-9
82740	Carcinoma rectum.....	-9
177	Carcinoma lung.....	+4
6977	Carcinoma stomach.....	-3
7088	Multiple myelomata.....	+4
7118	Carcinoma stomach.....	-12
92	Carcinoma cecum.....	-6
54	Carcinoma pancreas.....	-10
262	Carcinoma rectum.....	+6
391	Carcinoma gall bladder.....	+5
528	Carcinoma breast.....	+3
794	Melanocarcinoma skin.....	+4
1260	Carcinoma stomach.....	-2
1542	Sarcoma lung.....	+7
1573	Carcinoma stomach.....	+5
1659	General carcinoma.....	+4
1735	General carcinoma.....	+5
1563	General carcinoma.....	+6
1820	Sarcoma femur.....	+10
1761	Carcinoma colon.....	-4
1853	Carcinoma stomach.....	-5
1944	Carcinoma rectum.....	+7
2165	Carcinoma general.....	+9
2192	Carcinoma stomach.....	-11
2465	Carcinoma stomach.....	+10
3056	Carcinoma general.....	+4
2892	Carcinoma general.....	+10
3152	Carcinoma stomach.....	-3
2942	Carcinoma rectum.....	+4
3752	Carcinoma stomach.....	-5
3892	Carcinoma cervix.....	+5
4647	Carcinoma stomach.....	+7
5442	Carcinoma general.....	+4
P2386	Carcinoma stomach.....	-9
P2306	Carcinoma stomach.....	+12
P2488	Carcinoma sigmoid.....	+7
6368	Carcinoma bladder.....	+6
6479	Carcinoma stomach.....	+5
P2820	Sarcoma ovary.....	-5
6950	Carcinoma vagina.....	+3
831	Carcinoma bladder.....	-6
1235	Carcinoma prostate.....	+3

TABLE 4—Continued

CASE NUMBER	DIAGNOSIS	DISTURBANCE OF BLOOD SUGAR
		mgm.
1353	Carcinoma stomach.....	-5
1273	Carcinoma liver.....	+3
1587	Carcinoma stomach.....	-10
1825	Carcinoma stomach.....	-10
1869	Carcinoma esophagus.....	-6
2114	Carcinoma rectum.....	-6
2306	Carcinoma rectum.....	+10
2625	Carcinoma breast.....	-8
2709	Carcinoma generalised.....	-10
2471	Carcinoma generalised.....	-2
3604	Carcinoma cervix.....	+4
3517	Carcinoma breast.....	-6
3627	Carcinoma liver.....	+9
3021	Carcinoma esophagus.....	+6
3858	Carcinoma colon.....	+6
1740	Carcinoma esophagus.....	-6
3421	Sarcoma parotid.....	+4
3533	Sarcoma pelvis.....	+2
3836	Sarcoma retroperitoneal.....	+4
4197	Carcinoma stomach.....	-8
4213	Carcinoma breast.....	-5
4216	Carcinoma rectum.....	+8
225	Carcinoma stomach.....	+2
4510	Carcinoma stomach.....	-6
4134	Carcinoma stomach.....	+4
4414	Epithelioma cheek.....	-4
3097	Carcinoma stomach.....	+10
14149	Carcinoma stomach.....	+6
4747	Carcinoma stomach.....	+3
4565	Carcinoma stomach.....	-6
4493	Carcinoma stomach.....	-2
4708	Carcinoma larynx.....	-4
5113	Carcinoma stomach.....	+3
4748	Carcinoma cervix.....	+4
2590	Carcinoma intestine.....	+12
128	Carcinoma liver.....	-6
5193	Carcinoma cervix.....	-12
4531	Carcinoma tongue.....	-10
5821	Carcinoma breast.....	+12
6044	Carcinoma colon.....	+9
3225	Carcinoma breast.....	+10
5508	Carcinoma sigmoid.....	+8

TABLE 4—Continued

CASE NUMBER	DIAGNOSIS	DISTURBANCE OF BLOOD SUGAR
		mgm.
5836	Carcinoma stomach.....	-3
5839	Carcinoma breast.....	-9
158	Carcinoma stomach.....	+6
6153	Carcinoma esophagus.....	+6
754	Retroperitoneal sarcoma.....	-33
700	Carcinoma esophagus.....	-35
2300	Carcinoma stomach.....	-51
2315	Carcinoma lung.....	-51
3605	Carcinoma esophagus.....	+54
69	Sarcoma tendon sheath.....	+20
80	Carcinoma colon.....	+36
4936	Carcinoma stomach.....	-34
123	Epithelioma penis.....	-42
5637	Carcinoma uterus.....	-27
5793	Carcinoma uterus.....	+30
3728	Lymphosarcoma neck.....	+28
5746	General carcinoma.....	-34
6116	Carcinoma ovary.....	-33
6049	Carcinoma bladder.....	-31
6347	Carcinoma mouth.....	+30
1807	Carcinoma general.....	-21
1757	Carcinoma appendix.....	+19
2768	Carcinoma uterus.....	+22
3202	Carcinoma testes.....	-20
3560	Carcinoma stomach.....	+49
266	Carcinoma lip.....	+14
4965	Endothelioma brain.....	+30
594	Syphilis.....	-48
797	Inguinal hernia.....	-44
864	Encephalitis.....	-24
835	Biliary cirrhosis.....	-15
1102	Encephalitis.....	-16
1219	Syphilis.....	+21
1379	Neuritis.....	+42
770	Tuberculosis.....	+15
1580	Achylia gastrica.....	+15
1373	Gastric ulcer.....	-21
1299	Orchitis.....	+15
2222	Varicose veins.....	+18
2552	Leukemia.....	-42
2641	Fibroadenoma breast.....	+18
30	Cryptorchidism.....	-39

TABLE 4—Continued

CASE NUMBER	DIAGNOSIS	DISTURBANCE OF BLOOD SUGAR
		mgm.
31	Cryptorchidism.....	-20
32	Angioneurotic edema.....	-18
36	Hyperthyroidism.....	+28
37	Retinitis pigmentosa.....	+45
3209	Pernicious anemia.....	+28
3889	Pernicious anemia.....	+36
3822	Orchitis.....	-32
4464	Ovarian cyst.....	+29
2467	Endocarditis.....	+21
4792	Myocarditis.....	+28
4825	Cardio-nephritis.....	+48
4937	Lymphadenitis.....	+62
4943	Fibromyoma uteri.....	-40
5121	Endocarditis.....	+35
126	Gastritis.....	-36
5244	Postoperative adhesions.....	-18
5197	Pregnancy.....	-18
5356	Hypertrophied prostate.....	+34
5312	Fistula in ano.....	+16
5407	Catarrhal jaundice.....	+39
5452	Graves' disease.....	-33
5319	Pernicious anemia.....	-22
5645	Subacute gastritis.....	-24
5582	Catarrhal jaundice.....	-17
5628	Endometritis.....	-39
5654	Oophoritis.....	+24
5830	Neurasthenia.....	-17
5842	Gastric ulcer.....	+20
5814	Splenomegaly.....	-63
6115	Cholelithiasis.....	-22
6046	Mixed tumor parotid.....	+23
5406	Cerebral thrombosis.....	-53
6530	Diabetes.....	+60
6603	Uterine fibroids.....	+45
6841	Papilloma bladder.....	+22
237	Cholelithiasis.....	-42
3183	Diverticulitis.....	-15
527	Gastric ulcer.....	-28
583	Neurasthenia.....	+25
100	Tuberculosis.....	+16
1465	Ovarian cyst.....	+15
1109	Cholecystitis.....	+33

TABLE 4—*Concluded*

CASE NUMBER	DIAGNOSIS	DISTURBANCE OF BLOOD SUGAR
		mgm.
1639	Pyelitis.....	+22
1604	Cholecystitis.....	+18
1809	Sciatica.....	-15
1827	Chronic inflammation.....	-14
478	Gastric ulcer.....	+16
2554	Tuberculosis.....	+20
2588	Chronic cholecystitis.....	+18
2689	Gastric neurosis.....	+15
3229	Cholecystitis.....	-17
5436	Angina pectoris.....	-39
6843	Gastric ulcer.....	-38
5564	Lung abscess.....	-15
1511	Pernicious anemia.....	-9
35	Pregnancy.....	+6
5058	Gastric ulcer.....	+4
5372	Cystocele.....	+10
5401	Duodenal ulcer.....	+6
141	Orchitis.....	+10
5565	Syphilis.....	+10
5727	Cholecystitis.....	+
6016	Duodenal ulcer.....	+10
6063	Pernicious anemia.....	+9
6943	Chronic appendicitis.....	-7
7123	Nephritis.....	+11
136	Bronchopneumonia.....	-8
198	Fibroid uterus.....	+10
7086	Fissure in ano.....	+2
984	Prolapse uterus.....	+1
1008	Diverticulitis.....	+10
369	Duodenal ulcer.....	+2
1306	Fibroid uterus.....	+2
1873	Nephritis.....	-9
2298	Cholecystitis.....	+5
3121	Chronic endocervicitis.....	+4
3351	Cholecystitis.....	+3
3665	Tuberculosis.....	+4
3205	Hepatitis.....	-7
4440	Cholelithiasis.....	-5
4421	Polyp of sigmoid.....	+3
5570	Hypertrophy of prostate.....	+7
201	Septic endocarditis.....	+2
3566	Pernicious anemia.....	+9

cases of neoplasia of the gastro-intestinal tract and of the liver and gall-bladder show reactions of an intensity of less than 12 mgm., than is the case in individuals who have non-malignant diseases of these organs. In diseases of the genito-urinary apparatus this relationship does not apparently hold true, but in the series of miscellaneous neoplasias, no one type being present in sufficient numbers to warrant separate tabulation it is again evident that a larger proportion of the cancerous individuals show a lessened intensity of reaction than do a similar group of non-cancerous individuals.

TABLE 5

	REACTION	
	Less than 12 mgm.	More than 12 mgm.
Neoplasia of genitourinary tract.....	7 (54%)	6 (46%)
Neoplasia of gastrointestinal tract.....	50 (88%)	7 (12%)
Non-grouped neoplasia.....	29 (74%)	10 (26%)
Non-malignant disease of genitourinary tract....	9 (41%)	13 (59%)
Non-malignant disease of gastrointestinal tract ..	13 (38%)	21 (62%)
Non-grouped non-neoplastic diseases.....	9 (20%)	35 (80%)
All cases of neoplasia.....	86 (78%)	23 (22%)
All cases non-neoplastic.....	31 (31%)	69 (69%)

These differences are distinct when compared with the similarity of behavior found in our previous series where blood-sugar equilibrium was disturbed by the ingestion of glucose. The observation is possibly of value as a diagnostic procedure. In diseases involving the gastro-intestinal tract, from mouth to anus, duodenal ulcer excepted, and in diseases involving the gall-bladder and liver, 88 per cent of all the cases of neoplasia, i.e., 50 of 57 cases, showed a reaction of less than 12 mgm. As regards the reaction types it was found that 39 per cent of the neoplasia cases gave plus reactions as compared with 52 per cent in the non-cancerous group. If the previously outlined endocrine relationship be accepted, such lessened reaction would indicate either spleen, adrenal, or gonad insufficiency, or thyroid, adrenal, and pancreas overactivity.

CONCLUSIONS

1. The disturbance of blood sugar equilibrium which follows the injection of a protein varies in intensity and in type.
2. The removal of certain of the endocrines influences both the intensity of the reaction and its type.
3. Neither the intensity of the reaction nor the type have any relation to the strength of the antibody development after the injection of a selected protein.
4. A study of the type and reaction intensity has been used in an attempt to establish paths of endocrine correlation.
5. Human cases of neoplasia show a larger proportion of weak sugar reactions, i.e., of 12 mgm. or less, than do non-cancerous individuals. This is particularly true of neoplasms of the gastro-intestinal tract and liver.

REFERENCES

- (1) ROHDENBURG AND POHLMAN: *Am. Jour. Med. Sci.*, 1920, clix, 853.
- (2) ROHDENBURG AND KREHBIEL: *Am. Jour. Med. Sci.*, 1921, clxii, 28.
- (3) ROHDENBURG, BERNHARD, AND KREHBIEL: *Am. Jour. Med. Sci.*, 1920, clix, 577.
- (4) KILLIAN AND KAST: *Arch. Int. Med.*, 1921, xxviii, 813.
- (5) SPENCE: *Quart. Jour. Med.*, 1921, xiv, 314.

PREGNANCY AND TUMOR GROWTH

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The relationship between pregnancy and tumor development has been investigated by a number of observers, whose results have been, however, in many instances contradictory. For example, Moreau (1) and Herzog (2) found that transplanted tumors grow with unusual rapidity in pregnant animals. Haaland (3), on the other hand, maintained that pregnancy exerts a markedly restraining influence on the growth of transplanted tumors, which continue to develop in their usual fashion after parturition. Bashford and Murray (4) hold that "pregnancy" in the female "and full sexual activity in the male constitute no bar to successful transplantation." Uhlenhuth and Weidanz (5) observed retardation in the growth of tumors during pregnancy and also more frequent spontaneous retrogression. Bridré (6) noticed a low percentage of positive inoculations in pregnant animals, and Ehrlich (7) observed that inoculations into animals bearing young was frequently followed by negative results and that in those successfully inoculated the growth of the tumor was greatly retarded. Then, again, Albrecht and Hecht (8) maintained that pregnancy affects the growth of tumors just as little as the presence of a tumor influences conception or gravidity. Cuenot and Mercier (9) reported a most interesting observation. They noticed that Borrel's tumor "B," which rarely underwent spontaneous absorption, if inoculated before fecundation developed during gestation and receded during lactation. The tumor, however, did not regress if one mouse only was born, thus leaving the activity of the mammary gland at a minimum. Neither did absorption occur even in the presence of several young, if the tumor was so situated that its vasculari-

zation was independent of that of the mammary gland. Fichera (10) explained these various inconsistencies by assuming that when many embryos were present the food stuff was almost wholly consumed by them, while if there were only a few some of this material was available for the tumor cells.

Slye has concluded from her observations of spontaneous tumor growth in the mouse that: First, reproducing females grow much less tumor than do non-reproducing females of the same age, etc. Second, reproducing females grow much less tumor while they are reproductive than they do while they are non-reproductive. In other words, she finds that pregnancy exerts a markedly retarding influence upon growth of tumor.

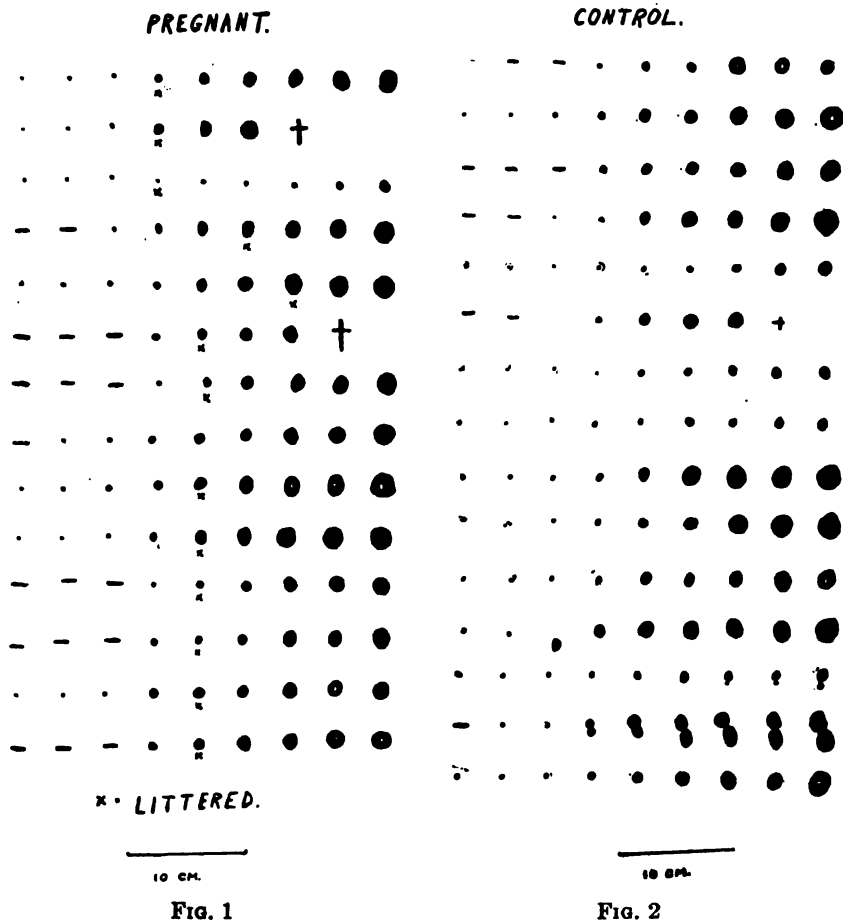
In re-investigating this problem rats were employed. The animals selected were young adults, sexually mature, vigorous, and in very good condition, which had been separated from male animals for a period of one month to avoid the possibility of unrecognized pregnancies. The rats were mated with strong, vigorous young males that were kept in the same boxes not only during the entire period of gestation but also after the females had littered, in order to impregnate the same animals immediately after parturition. In all cases the date of conception was calculated by counting back twenty-one days before the date of littering. All the animals were inoculated on the same day with 0.003 gram of Flexner's rat carcinoma, the time chosen being two days after the animals had been mated. In all, 105 animals were inoculated. Of these, 43 (41 per cent) became pregnant and littered normally.

In analyzing the cases in which no growth occurred, it is interesting to note that about 7.8 per cent of the males inoculated showed no growth, and that about 8.4 per cent of the females showed no growth. In the group of females without tumor growth, 44 per cent did not become gravid. The other 46 per cent did. These figures tend to show that in the negative cases neither sex nor gravidity played any rôle.

Tracings of the tumors were made every four days, and in this way growth was depicted simply but effectively.

A comparison of the charts (figs. 1 to 4) of the tumors of the pregnant and of the non-pregnant groups shows at a glance

that in these experiments pregnancy had absolutely no effect at all upon the growth of the transplantable Flexner rat carcinoma.



DISCUSSION

It seems impossible completely to reconcile all of the conflicting results which have been published in regard to the effect of pregnancy on the growth rate of inoculated tumors, chiefly because we have insufficient data upon which to base any conclusions

as to the growth capacity of the tumors which the various experimenters have employed. Nor do we know, in many instances, the site of inoculation. Mice are especially unsuitable animals for such a study, as the implanted tumors are apt to be

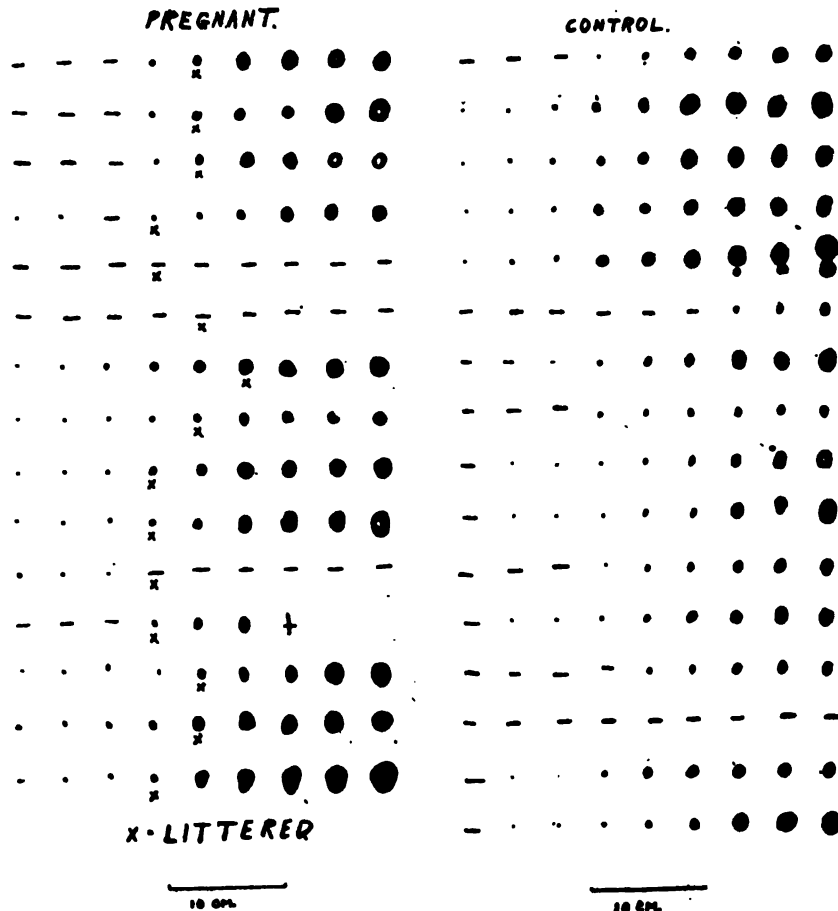


FIG. 3

FIG. 4

variable in their growth rate, and may become so large as to kill the animal quickly. This obviously complicates the problem and renders careful control and statistical analysis of the results necessary. But it does seem possible to draw a few conclusions,

not entirely inharmonious with the reported experimental work and the facts obtained by clinical observation.

The tumor grafts in the experiments here reported were not placed in the mammary gland but by intent in the axilla; they were, therefore, not affected by the increase in the vascularity of the breast at the beginning of pregnancy as they would be if placed directly in the main mass of the mamma. This increase in vascularity is undoubtedly the explanation of the rapid growth of tumors in the breast observed in human beings. But the conditions in the human female and in female mice are not comparable. A mouse weighing 15 to 20 grams will frequently carry a tumor weighing one-tenth of its weight. Unfortunately, the weights of the tumor-bearing animals are not given in Miss Slye's paper, though the dimensions of the tumors are furnished.

On the other hand, the weight of the tumor in a human female is rarely more than one one-hundredth or one one-hundred and fiftieth of the body weight. Mammary cancer in a mouse, therefore, may correspond in mass to a rapidly growing breast carcinoma of 10 to 15 pounds in a human female, a tumor which is practically never seen. It is quite possible that the demand for food for such a theoretical tumor if complicated by pregnancy might result in the starvation of the tumor for a time as Moreschi (12) has shown to be possible.

It is, therefore, evident that Miss Slye's results, while applicable to one species of mammalia under the conditions which she specifies, are by no means obviously transferable even to other types of rodents, much less to human beings.

The fact that in the additional experiments here reported no influence on the growth rate of tumors was shown is probably due to the disproportion between the size of the rat and its embryos, frequently weighing 150 grams with 25 grams of embryos, and the tumors, which are usually not more than one-fiftieth to one-hundredth of the weight of the animal. So, too, in mice the proportionate weight of the embryos demanding food is larger than that ordinarily occurring in the human female, a child rarely being over one-fifteenth of the mother's weight, while in the multiple mouse pregnancies 5 grams of embryos may be produced at a single birth from a mother weighing only 22 grams.

A grafted tumor, such as we have employed, being a mass of somatic cells growing in a foreign host, affords a possibility for studying the problem of tissue growth independently of organ or other relationships. The tumor is simply implanted in the subcutaneous connective tissue, while a primary tumor of the breast, in its inception at least, has important anatomical correlations with the tissue in which it grows. It is thinkable that some of the results observed by Miss Slye may be due to changes in the connective tissue stroma of the tumor rather than to the epithelium, but in the implanted tumor the connective tissue is furnished by the host, the epithelium being derived from another animal, so that the situation is quite different.

It seems safe to conclude, therefore, from our own experiments, that pregnancy of itself does not necessarily alter tumor growth rates, but that such interference, when it is observed, is due to the forced division of food substance between the tumor and the offspring; similar checking of the tumor can be observed in implanted tumors when the food of the non-pregnant animal is reduced approximately to the starvation point.

A comparable diminution in the tumor growth rate may be seen in human beings in the terminal stages of extreme cachexia with innutrition but, as a rule, in man pregnancy either has no effect on the progress of a cancer or, if the mammary or uterine tissues are involved, hastens the growth.

There is nothing, therefore, in the varying results of published experimental work which cannot be harmonized or which controverts the clinical observations already recorded.

REFERENCES

- (1) MOREAU: Arch. de méd. expér. et d'anat. path., 1894, vi, 693.
- (2) HERZOG: Jour. Med. Res., 1902, n.s. iii, 76.
- (3) HAALAND: Berl. klin. Wehnschr., 1907, xxiii, 718.
- (4) BASHFORD AND MURRAY: First Sci. Rep., Imperial Cancer Research Fund, London, 1904, p. 14.
- (5) UHLENHUTH AND WEIDANZ: Arb. a. d. k. Gandhtsamte., 1909, xxx, 440.
- (6) BRIDRÉ: Ann. de l'Inst. Pasteur, 1907, xxxi, 763.
- (7) EHRLICH: Verhandl. d. deutsch. path. Gesellsch., 1908, xii, 29.
- (8) ALBRECHT AND HECHT: Wien. klin. Wehnschr., 1909, xxii, 1783.
- (9) CUENOT AND MEROIER: Compt. rend. Acad. d. sc., 1909, cxlix, 1012.
- (10) FIGHERA: Jour. Exper. M., 1911, xiv, 320.
- (11) SLYE: Jour. Cancer Res., 1920, v, 25.
- (12) MORESCHI: Ztschr. f. Immunitätsforsch., Orig., 1909, ii, 651.

IS CANCER MORTALITY INCREASING?

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To determine whether or not there is a real increase in cancer mortality we must compare cancer death rates for the same ages, since cancer is an old age disease. The effect of age distribution is so great that it is safe to say that any considerable increase in the proportion of the population at the older ages, for instance, the emigration of the young men and women, would cause a noticeable increase in the cancer deaths per 100,000 population, while an influx of young men and women would decrease them.

Dr. Frederick L. Hoffman, statistician of the Prudential Life Insurance Company, in a paper on "The Menace of Cancer" published in 1913, and in his comprehensive book on "The Mortality from Cancer" published in 1915, has given the statistics available at the time of writing in a very complete form. These statistics were drawn both from the United States and abroad. From these I have selected a few.

In the registration of 1900, the cancer death rate per 100,000 population, which will hereafter be spoken of as the cancer death rate or cancer mortality, increased from 63 in 1900 to 79 in 1913, an increase of over 25 per cent. England and Wales for the same period showed an increase from 83 to 105 and most other European states showed a very considerable increase. Going back forty years, the rate in twenty large American cities was 49 for the period 1881-1885; for 1913 it had increased to 89. In twelve European countries the rate was 69 for the period 1896-1900; for the period 1906-1910, it had increased to 81. These were general population statistics without subdivision by ages. There were also available, however, some extensive statistics subdivided by ages. The cancer death rate in the registration states of the United States of 1900 subdivided into ten-year age groups, 25-34,

35-44, etc., for 1901 compared with 1911 shows an increase in the rate for each age group for the later year, such increase being very considerable for ages above 45, which are the significant ages in cancer. Massachusetts, for the two periods 1901-1905 and 1906-1910, with a different distribution into age groups and subdivided by sexes, showed an increase for each adult group and for each sex separately, in the later period the increase again being very considerable for the older groups. While these are but a few of the statistics, they are illustrative of the trend of practically all, and, standing alone, would appear to indicate a great and rapid increase in cancer mortality. We have, however, later and very extensive statistics from two of the great insurance companies which are not in accord with the above.

In 1919, Dr. Louis I. Dublin, statistician of the Metropolitan Life Insurance Company, published in this JOURNAL (1) the experience of that company on its millions of industrial policy holders for the years 1911-1916. Tables 1 and 2 are from this experience. They show fluctuations but no definite trend.

Table 1 is for the age group 55-64 only, which group Dr. Dublin chose as illustrative. We find in it the lowest mortality for white males in 1912 and 1915, the highest in 1913 and 1914; for white females the lowest in 1911 and 1914, the highest in 1912 and 1915. It is a curious accident that the years of low mortality for the one sex are in three cases the high for the other.

Table 2 compares the first two years of the period with the last two and in total shows but one per cent difference between them, a difference smaller than would be expected from merely accidental fluctuations. The two features which may be of significance are that the total of males shows an increase of 5 per cent and that the differences in each age group, as well as in the total of all ages (except perhaps 65-69), are no more than would be expected from accidental fluctuations.

The cancer experience for the Mutual Life Insurance Company for the years 1915-1920 has just been compiled by Dr. Brandreth Symonds, chief medical director. This experience, given in table 3 below, is not subdivided by sexes, but as the great preponderance of insurance was on white male lives it may be taken as representing white male cancer mortality.

TABLE 1
Metropolitan Life Industrial Department. Cancer death rate per 100,000,
ages 55 to 64

YEAR	ALL CLASSES	WHITE		COLORED	
		Males	Females	Males	Females
1916	386.4	358.0	427.4	218.3	339.9
1915	380.8	336.0	427.8	175.7	394.3
1914	390.9	385.0	423.3	167.7	351.7
1913	384.1	370.3	414.6	195.2	368.3
1912	381.9	334.1	443.2	176.4	325.4
1911	368.7	353.3	400.2	158.0	373.7

TABLE 2
Metropolitan Life Industrial Department. Ratio of cancer death rate for years
1915-1916 to death rate for years 1911-1913 by percentages

AGE PERIOD	ALL CLASSES	WHITE		COLORED	
		Males	Females	Males	Females
	per cent	per cent	per cent	per cent	per cent
25 and over	101.0	105.2	98.3	105.1	100.7
25 to 34	98.0	121.0	94.1	95.5	95.0
35 to 44	100.1	104.3	96.9	142.6	98.2
45 to 54	99.1	109.7	97.0	67.7	93.1
55 to 64	102.2	101.1	101.2	118.0	105.2
65 to 74	107.0	115.9	101.9	117.2	107.0
75 and over	101.0	104.1	104.4	110.7	48.6

TABLE 3
Mutual Life Insurance Company. Cancer death rate per 100,000

AGE PERIOD	1915	1916	1917	1918	1919	1920	ALL YEARS 1915-1920
25-29	15.34	3.98	0.90	7.53	1.47	5.08	5.35
30-34	11.52	15.19	19.55	10.54	5.52	5.49	10.55
35-39	7.61	12.95	13.38	10.99	5.74	18.69	11.87
40-44	31.39	45.31	30.86	26.92	33.73	21.21	31.07
45-49	40.36	41.82	51.59	60.96	51.56	78.80	55.15
50-54	165.47	157.35	100.67	79.25	73.45	70.02	105.78
55-59	132.22	213.84	204.08	153.07	152.73	159.95	168.88
60-64	235.74	366.73	255.24	308.53	285.56	272.73	287.21
65-69	225.77	380.94	532.84	357.91	502.42	423.88	407.99
70 and over	554.09	1000.67	834.83	856.57	731.75	982.20	829.54

If the first two years, 1915-1916, combined are compared with the last two, 1919-1920, we find that up to age 65 the cancer mortality ran in general considerably lower for the later than for the earlier period, while for ages 65 and older the reverse was true. Of the nine five-year groups (including "70 and over" as such a group), beginning with age 30-34, five show a higher mortality in the earlier two-year period and four in the later. Had the subdivision into age groups been the ten-year groups of the Metropolitan, beginning with 25-34, every group up to age 65 would evidently have shown a higher mortality in the earlier period.

The year 1915, for some unexplained reason, was a year of very low cancer mortality in the Mutual, a peculiarity which did not appear in the Metropolitan experience and which was, therefore, probably accidental. It is consequently worth while to see what would have been the result had 1915 been eliminated so that we should have compared the years 1916-1917 with 1919-1920. We find that of the nine five-year groups, seven would have shown a higher mortality for the earlier period and only two for the later; moreover, had the grouping been by ten-year groups, as in the Metropolitan experience, each ten-year group from age 25 up would evidently have shown a higher mortality in the earlier period than in the later.

Again, a comparison of the last two years combined with the preceding four years combined, shows that of the nine groups five had a higher mortality in the earlier period; we must recognize, however, that two of the four were 65-69 and "70 and over" which are of greater importance than groups near the lower age limit. Had the exceptional year 1915 been eliminated, however, the result would have again looked extremely favorable for the later period.

In order to have the most recent possible data, the approximate cancer mortality for the year 1921 has been worked out, although exact figures are not yet available. The 1921 results compared with the average for the preceding six years show a lower mortality for each of the seven five-year age groups from 30-34 to 60-64, but a higher mortality for 65-69 and "70 and over."

Emphasis is given to the failure of the Mutual experience to show any tendency to an increasing death rate by the fact that the lives involved were nearly all white males, and in other experiences where the sexes are separated any tendency towards increase has generally been more among males than females.

The Metropolitan experience has been extended from 1915 through 1920 but has not yet been made public. Through the courtesy of Dr. Dublin I have had an opportunity to see these statistics and to include in this paper the deductions from them. They show in general the same lack of any tendency to increase in the age groups from age 30 to age 65, but they show an increasing tendency from age 65 upwards.

Thus, we have had the experience of the two companies covering the eleven-year period from 1911 to 1921, inclusive. The data in these experiences should probably be at least as accurate as to the causes of death as those of any other experience, because the insurance companies try to obtain accurate knowledge of the cause of death at the time the claim is paid. The figures indicate, taken at their face value without considering whether there are any modifying influences, for ages below 65 either a fluctuating or a slightly decreasing cancer mortality; for ages 65 and over they vary, but probably, on the whole, indicate some increase.

The above are the direct deductions from the statistics. Before accepting these as final results, however, we must go back of the statistics and inquire whether there are any circumstances or considerations relating to the data on which these statistics are based tending to modify the results. One such consideration should be mentioned. With the development of medical science there has been a gradual increase in correctness of diagnosis of the cause of death. Professor Walter F. Willcox (2) showed in 1917 that a very considerable apparent increase in cancer mortality would result simply from the continued improvement in the correctness of diagnosis, which increase in correctness had probably continued up to the present day. Undoubtedly in the past many deaths attributed to old age should properly have been set down as cancer, and many others attributed to other causes would undoubtedly have been attributed to cancer if the correct diagnosis had been made. The result of this would be

that if there were a really stationary cancer mortality it would, nevertheless, appear to be increasing considerably because of the increasing correctness of diagnosis.

How effective this could be to cause an apparent increase where no real one exists may be seen in the statistics of appendicitis mortality which show an increase of 40 per cent from 1900 to 1915; yet undoubtedly the real rate was decreasing in this period because of surgical advance and readiness to resort to surgery.

Moreover, increasing correctness in diagnosis works almost altogether in one way; that is, it results in attributing to cancer many deaths which would formerly have been incorrectly attributed to some other cause, and in changing but few the other way. The effect of increased correctness in diagnosis, which is undoubtedly more important at the advanced ages, would seem to me enough to explain what apparent increase in cancer mortality there is in ages above 65, and it would emphasize the fact that for ages below 65 there has not been even an apparent increase.

Meanwhile the statistics of the United States Registration Area show a continuous increase in cancer deaths. These are population statistics, however, and are not analyzed as to ages; hence, as compared to such statistics as we have considered, they have very little meaning, since a change in the age proportion of the population might be responsible for the entire apparent cancer increase.

My conclusion would be, even taking into account the registration area's apparent increase, that we cannot now determine whether the cancer mortality is slightly increasing, practically stationary, or slightly decreasing, but that we can be sure it is not greatly increasing. A more exact result is something for future investigations when reliable statistics for a long period of years are obtainable.

Lest what I have said be misinterpreted I would add that such a conclusion does not lessen at all the seriousness of the cancer problem. It merely holds out hope that the terrible scourge will not increase without limit.

REFERENCES

- (1) DUBLIN, L. I: Mortality Statistics of Cancer Among Wage Earners, etc., J. Cancer Res., 1919, iv, 235.
- (2) WILLCOX, W. F: On the Alleged Increase of Cancer, Trs. Am. Statistical Assoc., 1917, xv, 701 and J. Cancer Res., 1917, ii, 267.

CANCER AND PARASITE

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Ever since the first recognition of the bacteria as a cause of disease, attempts have been made to demonstrate a bacterial cause for malignant tumors. The importance of such a discovery, with all its possibilities of prophylaxis or even cure, is obvious, and accounts for the sustained interest in this question, in the face of constantly repeated failure.

One of the most recent investigators is Nuzum (1) who employed a mouse carcinoma (no. 11) from the Crocker Institute. From this growth he isolated a diplococcus and described a malignant tumor having all the characteristics of carcinoma 11, which followed inoculation of a culture of this organism into mice.

The importance of such a discovery would be, needless to say, beyond computation, and it was therefore determined to repeat the experiments with additional checks and controls. That microorganisms are frequently found in malignant tumors, especially those growing in mice, is a well established fact, but, as has been said, no one has ever succeeded in proving that they play any rôle as causative agents.

To summarize Nuzum's findings, it will suffice to say that he cultivated pieces of tumor in tissue ascitic fluid media covered by paraffin, and thus partially anaerobic. From this culture he isolated a Gram-positive diplococcus, which he considers the specific bacterium in the production of carcinoma 11. A subculture was made of this organism in the same manner, and inoculated into mice. In three of them, the typical carcinoma 11

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is said to have developed. However, careful perusal of the article suggests that only two of these mice had a real tumor.

As can readily be seen, two factors must be considered in an experiment of this nature: (1) the tumor cell proper; and (2) the microörganism, if any. In order to test the ability of the latter to produce the malignant growth in question, it is necessary to prevent any participation of the tumor cells in the process. To do this without affecting the microörganism, two methods were employed in my own experiments, radiation and freezing.

In the first, several mice that had well developed carcinoma 11 tumors, were exposed to the Roentgen ray, being given a dose known to destroy the tumor cells completely. The rayed tumor was then excised aseptically and inoculated into 24 mice in the usual fashion. Part of this same tumor was cultured, following most minutely the technic laid down by Nuzum. As a control, carcinoma of the same series, from another mouse, was inoculated into 24 mice, and fragments were put at the same time into twelve tubes of the ascitic tumor fluid media. The tumor used in this control series was not radiated.

Transplantation of the unirradiated control tumor resulted in the usual 60 per cent of takes which this tumor has given during the past seven or eight years at the Crocker Laboratory. On the other hand, in not a single instance did a tumor develop from the radiated cells. In other words, where the tumor-cell factor was eliminated the microörganism factor, which was being tested, was not able to reproduce the tumor.

Because of the extreme importance of the question, this experiment was repeated in another series of mice, with the same negative results.

The cultures made from the radiated tumor were incubated for five days and were then inoculated into 60 mice. In none of the 60 did a tumor subsequently develop. At the same time, smears were made from the bottom of the culture tubes and stained with Gram's and with Giemsa's stain; these contained in most instances many short and long bacilli, streptococci, staphylococci, and diplococci. In some smears all these varieties were present and most of the smears, in fact, showed more than one

organism. In no instance was a pure culture of any one organism found.

The second method employed to eliminate the tumor cells was alternate freezing and thawing of the tumor before inoculation. The growth was removed aseptically and placed in a sterile dish, where it was emulsified with scissors, and the dish was then placed in an ice and salt mixture. After about fifteen minutes, when the mass was frozen solid, it was ground with a sterile pestle until it became soft and fluid. This freezing and thawing was repeated twice, with the purpose of destroying the tumor cells without harming any bacteria that might be present. Part of this emulsion was inoculated into 36 mice, and another part was cultured in the ascitic tissue fluid media.

In only two of the 36 mice was there any growth, and in both instances the tumor was not discoverable until the twenty-eighth day after inoculation, whereas in routine laboratory inoculation, growth is generally noted in from seven to ten days. Hence the freezing and thawing had killed all but a few of the tumor cells. If carcinoma 11 were due to a microorganism, all, or at least most of the mice, should have had tumors, both in the frozen and thawed tumor series, in the rayed tumor series, and in the culture inoculation series. The absence of a tumor in all but two late instances, militates against the assumption that carcinoma no. 11 can be ascribed to a microorganism, especially in view of the fact that no one type of organism was found as a constant occurrence. The two growths that did develop from frozen and ground tumor can be readily explained by the assumption that the freezing did not destroy quite all the tumor cells. Others of the staff at the Crocker Laboratory have had the same experience.

In all the cultures the results, as concerns the specific organism described by Nuzum, were negative as has been already stated and subcultures inoculated into 24 mice produced no tumors.

A careful perusal of the experiments of Nuzum leads to the conclusion that in only two instances out of eighty-nine did the culture fluid inoculation cause tumor growth, i.e., animal no.

250 in experiment 1, and a second animal in experiment 3. These two instances and our own negative findings repeatedly and carefully checked up suggest that in all probability the tumors in Nuzum's experiments were spontaneous new growths.

It may be stated in conclusion that all the animals showing negative results were kept under observation for a period of three and a half months, so that ample time was given for a tumor to arise.

CONCLUSIONS

1. While most of the ascitic tissue fluid cultures of Crocker Institute carcinoma 11 contained microorganisms of various kinds, in no instance was there found the characteristic microorganism described by Nuzum.

2. In not a single instance was the inoculation of mice with these cultures followed by tumor growth.

3. It is probable that the two undoubted tumors in Nuzum's series were a spontaneous new growth.

REFERENCE

- (1) Nuzum: Surg. Gynec. and Obst., 1921, xxxiii, 167.

TERATOMAS AND THEIR RELATION TO AGE

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The great accumulation of reported cases of teratomas offers an excellent opportunity to review the data, with the object of investigating the relation of their frequency to the age of the host. In the course of the study, it has become apparent that this relationship is so definite as to assume the form of a general law.

The largest collections of teratomas were gathered by Taruffi and Ahlfeld. Both of these observers stressed the large congenital forms which are situated in the head, the thoracic, the abdominal, or the sacral regions. Gonadal teratomas occur most frequently in early adult life (Wilms). Among others, Askanazy investigated the internal craniopagi, and Ekehorn, the internal thoracopagi. Lexer and Nakayama studied the abdominal inclusions and pygopagi.

Though many hypotheses have been advanced on the origin of teratomas, they may be resolved into two view points: The teratoma is either the offspring or the twin of its host. Stockard has recently produced experimental evidence in favor of the latter conception.

CRITERIA AND METHODS OF STUDY

Two precautions have been observed in compiling the present statistics: (1) Only growths of tridermal or bidermal origin have been considered. No such case has been omitted. (2) Special effort has been exerted to determine the age at which the teratoma began its growth. The first increase in size of external growths may be accurately observed. For those

situated internally, the initial symptom was used as an indicator. Where the history was deficient, the age at which the operation took place or at which death occurred was taken as the closest approximation obtainable. The last criterium particularly applies to the teratomas of the aged. In this manner 895 cases have been studied. Sometimes a period of slow growth is followed by one of heightened activity. Such is the case of chorioma testis reported by Jackson, in which growth commenced at the age of twenty and slowly continued to twenty-three, after which the increase in size became extremely rapid. Since the relationship between the growth of the host and that of the teratoma is of interest, in such cases, the beginnings of both periods have been noted. A similar effort was made for internal teratomas, thus bringing the total number of growths tabulated to 975.

In systematizing the results it was noted that though the variation from year to year is considerable, there seems to be an orderly waxing and waning of the number of cases to an extent which justifies the drawing of a curve. An average has been drawn in order to minimize accidental variation. Six year periods have been chosen because they are the longest which correspond to actual changes throughout the length of the curve. The first period begins at fertilization and ends at five. In the curves which are drawn to a scale of one-half, the abscissae represent the age of the host when the tumor began its growth, the ordinates the number of cases in each year.

THE CURVE FOR ALL TERATOMAS

After an initial maximum rise, the curve falls and remains low from five to eleven years (fig. 1). At eleven it achieves a higher level, which is increased at seventeen and twenty-three years. The second maximum is found between twenty-three and twenty-nine years. The curve falls gradually at twenty-nine and thirty-five years, and then more rapidly at forty-one, after which it becomes progressively lower towards its end at seventy-six years. Eighty-seven per cent of the teratomas occur before forty-one years and 95 per cent before fifty-three.

Most congenital teratomas do not evince postnatal growth and since this study concerns only those which do grow, for the early maximal total may be substituted the smaller number of tumors, showing power for growth, represented by the lower broken line in the graph. With this correction the highest point in the curve is found between twenty-three and twenty-nine years. This is borne out by Wilms who finds the period of greatest frequency for sex-gland teratomas to be between the ages of twenty and thirty years. His conclusion is to be expected because teratomas occur most frequently in sex glands.

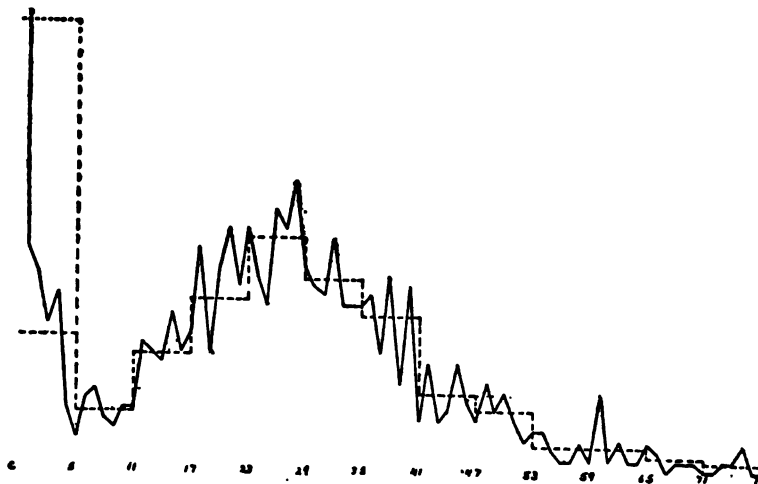


FIG. 1. CURVE FOR ALL TERATOMAS

The abscissae stand for the years of appearance of growths, the ordinates for the number in each year. Both are drawn to a scale of one-half. The total number of congenital tumors is not shown. The dash line indicates the average for each six-year period. In the first six years there are two; the upper one stands for all cases, the lower for those which had power of postnatal growth. The mode is from twenty-three to twenty-nine, the time when growth stops.

STUDY OF THE CORRECTED CURVE

There are three aspects of the described phenomenon: First, it is evident that the total number of teratomas at any age increases with the actual growth of the individual. As size

increases, the total number of teratomas increases. But it must be remembered that while size is increasing, growth rate is falling. Therefore, second, teratomas become more frequent as growth slows down, at the time when growth potential becomes smaller. Growth must be recognized as involving two elements: increasing actual proportions, and decreasing growth potential. Hence, third, the total number of teratomas at any age increases as growth potential diminishes.

When one recalls the fact that the changes in growth rate are not constant, the number of teratomas is seen to bear even a closer relationship to the growth of the hosts than has been indicated. Not only do these tumors appear as growth of the host slows, but during their time of appearance the teratomas are more frequent in the periods of slower growth of the host. Teratomas are common in early infancy following a space of the most rapid proliferation of all—fetal growth. They increase again when the comparatively rapid growth rate of early childhood gives way to the slower one of pubescence and lastly they are found in greater numbers as active growth gradually ceases.

To explain the relative number of teratomas appearing in the several periods we must take into consideration an additional factor, the growth potential of the embryonal rest, for a teratoma by most theories arises in an embryonal rest of some kind. It is known that the great majority of embryonal rests do not grow; they either degenerate or remain dormant. Others achieve a more or less perfect adult growth, while a few develop into tumors. Tridermal rests act in a similar manner. Thus we have a few with high growth potential, many with less capacity for growth, and finally others which remain latent unless they are stirred to development by an external stimulus.

During the first six years of life the growth rate of the host declines rapidly, his growth potential is greatly reduced, and the number of teratomas of relatively higher potential, capable of proliferation, is comparatively large. From five to eleven years the growth rate is fairly constant, the loss of potential is small, and the additional number of teratomas released is few. The next appreciable change takes place during the differentia-

tion occurring at puberty and accompanying the maturation of the sex organs. Here the number of teratomas begins to increase and continues to do so until growth finally stops, at which time the greatest number of teratomas make their presence known. Although the loss of growth potential in the host becomes smaller, being least in the final period from twenty-three to twenty-nine years, yet just because growth itself is slow, there is an ever increasing number of embryonal rests of low potential, capable of expressing their latent growth energy. The very inactive rests even at this period do not have sufficient energy to start growth spontaneously and are therefore considered in another group at a later time. However, they may serve as a nidus for neoplasms since many do commence development after the growth of the host has stopped.

Thus the number of teratomas appearing at any given time depends upon the amount of loss in growth potential of the hosts during that time and the number of rests whose potential is large enough to proliferate under these conditions. Evidently *the number of teratomas in any period varies inversely to the growth potential of the hosts and directly as that of the embryonal rests.*

ANALYSIS OF CURVES OF AGE INCIDENCE OF TERATOMAS IN THE DIFFERENT LOCATIONS

The object of this study is to show the relation to the general law of the occurrence of teratomas in the various situations.

Thoracopagi. Ekehorn in his collection of teratomas of the anterior mediastinum finds fifteen cases occurring between the ages of twenty and thirty, four between thirty and forty years, and four more to sixty, thus agreeing with the present curve in showing the maximum occurrence from twenty-three years to twenty-nine.

Abdominal inclusions. The abdominal tridermal growths are frequently discovered at birth but continue to be found throughout life, with a second rise from twenty-three to twenty-nine years.

Craniopagi and pygopagi. There is not a sufficiently large number of head and sacral teratomas to yield reliable statistics.

Statistics of various authors. They show a close agreement with original statistics here presented. Note increased number of dermoids during pubescence

THORACOPAGI (CHRISTIAN)		DERMOIDS (PAUL)		EMBRYOMES (CHEVASSU)		SEMINOMES (CHEVASSU)		CARCINOMAS		
									Testis	Ovary
10-20	1	1-5	4	0-5	5	0-19	0	0-5	2	11
20-30	19	5-10	3	6-17	0	20-25	1	5-11	2	25
30-40	7	10-15	10	18-24	16	25-30	8	11-17	1	48
40-50	2	15-20	8	25-29	11	30-35	8	17-23	12	19
50 +	2	20-25	12	30-34	11	35-40	19	23-29	29	26
		25-30	14	35-39	7	40-45	14	29-35	30	21
		30-35	10	40-44	3	45-50	4	35-41	34	16
		35-40	11	45-49	4	50-60	2	41-47	27	16
		40-45	8	50-59	2	60-63	2	47-53	10	14
		45-50	10	60-75	2			53-59	8	14
		50-55	5					59-65	1	1
		55-60	2					65-71	1	
		60-65	1							
		65-70	2							
		70 +	2							
	31		103		61		59		157	207

Inclusions according to age and situation. Those which are capable of growth appear in greater number from twenty-three years to twenty-nine years

AGE	CRANIOFAGI	THORACOPAGI	ABDOMINAL PARASITES	OVARIAN TERATOMAS	TESTICULAR TERATOMAS	PTGOFAGI	TOTAL
-5	78	55	36	17	48	45	279
5-11	1	3	6	15	7	1	33
11-17	4	11	18	27	9	1	70
17-23	4	14	16	44	32	4	114
23-29	1	16	20	52	55		144
29-35		10	15	47	44		116
35-41		4	8	39	43		94
41-47		4	4	22	15		45
47-53		4	4	19	7	2	36
53-59		1	1	13			10
59-65		1	2	10	3		16
65-71			1	6	1		8
71-77	2		1	2			5
Total. ...	90	123	132	313	264	53	975

Most are congenital. The internal craniopagi occur most frequently from eleven to twenty-seven years. The only year where there is more than one case is the nineteenth, where there are two. The last teratoma capable of spontaneous growth appeared at twenty-seven. The later examples of sacral teratomas are even rarer than those occurring in the head. There is one each at ten and thirteen years and four from nineteen to twenty-three.

Ovarian teratomas. Turning next to the gonadal teratomas and comparing the testicular and ovarian curves, we see that the latter is less variable (fig. 3). This is due to the delayed diagnosis of so many of the ovarian teratomas, probably because of their slow growth, as their structure is often of the adult type. Their internal position further postpones their discovery, yet dermoids are sometimes found by accident. Nevertheless, the largest number of tumors occurs between the ages of twenty-three and twenty-nine. From a review of one hundred and three cases Pauli finds dermoids appearing most often from twenty to thirty years.

Another difference between the ovarian and testicular curves is the greater rise in the former in the two periods between eleven and twenty-three years. This phenomenon might be expected as a result of the growth differences since, in the male, postpubescent growth is more rapid than in the female.

Teratoma testis. The external situation of the male sex gland allows prompt discovery of its tumors. Although the greatest decline in rate of growth occurs early in life from birth to four years and the number of testicular teratomas at this time is large, the maximum number nevertheless occurs at a later period. This is probably due to the fact that growth under four years is comparatively very rapid in spite of its fast declining rate. The modal year of the curve is twenty-six. It is interesting to note that the last growth cartilage of the long bones ossifies at twenty-five.

Teratomas in the testes commence growth most frequently between the ages of twenty-three and twenty-nine years. Chevassu finds the maximum between twenty and thirty years (fig. 2).

TERATOMAS OF LATER LIFE

After establishing the time of greatest frequency of teratomas, it still remains to account for those of old age. Those diagnosed after the age of fifty-one may be divided into two groups. The first consists of neoplasms of adult structure which had reached the limits of their capacity for post-natal growth, while those of the second are more malignant. To the first group belong such tumors as the following:

Craniopagi. Beck reports a case in which a dermoid was found at autopsy in place of the hypophysis in a woman seventy-four years of age. Eberth reports a similar accidental finding beneath the dura in a woman of seventy-five.

Thoracopagi. There are two examples in Ekehorn's collection—Pinder's case of a patient with bulbar paralysis, aged fifty-three, in whom the dermoid was discovered at autopsy; and Lebert's of a man of sixty who had been dyspneic since his sixteenth year.

Abdominal inclusions. Rizzoli (Taruffi) reports two cases of late abdominal inclusions, one at sixty, the other at sixty-two. Symptoms had been present for a long time in both. In one of the cases they appeared first at the age of twenty.

The tumors mentioned thus far were benign, though some produced symptoms because of their size and position.

In the second group of neoplasms the element of trauma becomes important in the etiology. There is Bonney's report of a retroperitoneal chorioma of a man of sixty-seven, and Goebell's of an abdominal teratoma that became malignant at fifty-four, twenty-seven years after a mass had been diagnosed. Djewitski reports a chorioma of the bladder which first gave symptoms at the age of seventy-three. The same irritation which produces a papilloma of the bladder may transform an otherwise benign embryonal rest.

Pygopagi. Hudson describes a sacral teratoma which began growth at the age of fifty-two; the history shows that a nodule had existed in that region since the birth of the patient. The histological picture is one of a tridermal rest with cancerous degeneration of mucous glands. It is similar to an old age

cancer arising in previously normal tissue. Evidently in the last four cases it is not growth potential of the embryonal rest but an extrinsic traumatic influence, to which every part of the body is subject, that caused the proliferation of cells. A case of Briddon's beautifully illustrates both these factors occurring in the same growth but independently and at different times. It concerns a sacral dermoid which appeared externally at the age of twenty-two and then ceased growth till the fifty-second year, when it underwent epitheliomatous change.

Teratoma testis. There are three examples of late teratoma testis. Lexer quotes one from v. Bergmann's clinic in a man of sixty. On section the growth was of adult structure.

Ewing and Pepere report cases which first showed growth at the ages of sixty-one and sixty-three, respectively. The microscopic examination in both instances showed carcinomatous change of one element in a totipotent rest.

Ovarian teratomas. Of thirty-two dermoids, thirteen exhibited malignant transformation of a carcinomatous, sarcomatous, or endotheliomatous type. Four showed thyroid structure, of which three were rapidly growing tumors. In six reports details were lacking. However, since the growths were called dermoids, their structure must have been of the adult type, like that of the remaining nine inclusions.

To summarize, the late appearance of stationary teratomas is due to their delayed discovery, while that of growing teratomas is caused by their injury.

Carcinoma testis. Carcinoma testis is discussed in this place not only for its possible teratomatous origin, but because traumatic etiology links it with the tumors of later life. In many cases of teratoma testis in young people the transformation of a slowly into a more rapidly growing tumor is caused by trauma. In older people the growth is rapid from the start. The same sequence of events obtains for carcinoma testis. The cell of many cases of carcinoma testis is characteristic, with a large nucleus and clear cytoplasm. Sometimes the growth is called a sarcoma; the difference in opinion is due to the fact that no analogous cell is found in the human body. This same cell is

often found with teratoma testis. There are only two probable interpretations: (1) The irritation caused by some extrinsic factor, in this special case, by the teratoma on the tubule cells, is the cause of carcinoma. (2) The unique type of cell is of teratomatous origin. Chevassu takes the position that it develops from the adult spermatogonia, putting the tumor in the class of acquired carcinomas. The final convincing link in the chain of evidence has not been produced, for he has not been able to trace the steps of anaplastic change from the spermatogonia to the carcinoma cell.

It has been definitely established that the cell which is of more rapid growth will often overrun and may finally crowd out altogether the other constituents of the tumor. Thus arise the rhabdomyomas, the chondromas—the simple tumors of the sex glands. In this uncontrolled competition the most embryonal type of cell would have a decided advantage. Therefore Ewing concludes that carcinoma testis is a one-sided teratoma. In the light of the foregoing it is interesting to see to which of these two theories the carcinoma curve lends itself.

According to Chevassu's statistics embryomas occur with greatest frequency from twenty-five to thirty years and seminomas from thirty-five to forty (fig. 2). The writer's review of a larger number of cases coincides with the data of Chevassu, the modes occurring from twenty-three to twenty-nine years in the teratomas and thirty-five to forty-one years in the carcinomas.

Comparing the carcinoma testis curve with that of all cancer (Hoffman¹), of which the congenital cases are too small a propor-

¹ Mortality from cancer throughout the United States Registration Area. All organs and all parts. 1903-1912. (Hoffman, *The Mortality from Cancer.*)

	MALE	FEMALE
Until 10	1, 170	984
10-24	2, 028	1, 844
25-34	3, 757	7, 891
35-44	10, 750	26, 779
45-54	24, 431	46, 669
55-64	35, 327	52, 393
65-74	33, 745	43, 010
75 and over	18, 381	24, 601

tion materially to alter the general outline, we see that the former has no resemblance to the latter, for in that case it would have a continuous rise to some time after sixty. In brief, the carcinoma testis curve is the teratoma testis curve with the mode slightly shifted.

Since the histogenesis of carcinoma testis has not been traced from either embryonal or adult tubule cells, it is probable that carcinoma testis is of nontesticular origin, and since there is no reason why misplaced cells should so often be of the same type or occur so frequently with teratomas, unless they are of tera-

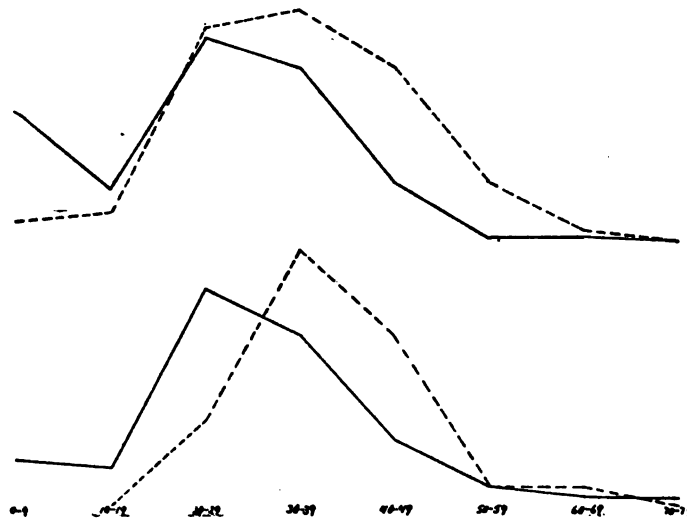


FIG. 2. TESTICULAR TERATOMAS

The lower two curves are reproduced from Chevassu's paper. The continuous line represents the teratomas; the dash line, the carcinomas. In order better to compare the new curves with those of Chevassu, the number of teratomas in each year was divided by four, and that of carcinomas by two. Both curves are plotted on a basis of ten-year periods.

tomatous origin, we are forced to this conclusion towards which the study of the curve gives additional evidence. Similarly to teratoma testis, carcinoma may show a congenital increase in the size of the organ. Morestin (Chevassu) reports such a tumor, which assumed malignancy at the age of thirty-seven. Like-

wise carcinoma occurs more often in undescended testicles. It begins its growth in the rete and, like teratoma testis, is occasionally observed in pseudohermaphrodites.

Ovarian tumors. The origin of primary tumors of the ovary is so undecided that any data in reference to them is of particular interest. Here we shall mention a few facts in regard to one of these tumors which may be of teratomatous origin, i.e., the sarcoma.

Ewing divides these sarcomas into three main types: (1) spindle cell; (2) round cell; (3) myxoma cell. This classification is of special significance since just such types of sarcomatous degeneration of dermoids have been observed (Debucy).

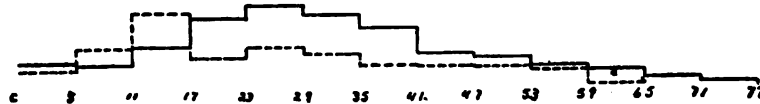


FIG. 3. OVARIAN TERATOMAS

These curves are averages for six year periods drawn to a scale of one-half. The continuous line represents the teratomas with the mode from twenty-three to twenty-nine years; the dash line the carcinomas with the mode from eleven to seventeen years, the period of pubescence.

Desurmont finds that the different primary tumors are bilateral to varying degrees. However, sarcomas, 25 per cent, and dermoids, 20 per cent (Pauli), approximate each other quite closely.

Finally, the most common tumor of infancy is the sarcoma, which is most frequent at fifteen years (Donhauser). Cordier and Zangemeister give fifteen and twenty years, respectively, as the age of most common occurrence of sarcomas. They are found from fifteen years to twenty-five and from forty years to fifty (Desurmont), both periods of physiological stimulation. The writer finds the mode of the combined carcinoma and sarcoma curve at fifteen years (fig. 3). Comparing the mode of this curve with that of ovarian teratomas, we find that it has been shifted forward to the time of pubescence. Hence there is a group of embryonal cell tumors having an age incidence similar to teratomas, and becoming malignant under the stimulation of puberty.

FACTORS IN THE ETIOLOGY OF TERATOMAS

The growth of the earlier teratomas may be adequately explained on the basis of a growth competition between the host and the embryonal rest. But even in the teratomas of infancy another factor, trauma, may be present. It becomes increasingly important later on.

Growth potential. The growth of the host inhibits that of the teratoma.

The growth of the embryonal rest may be divided into two parts: its prenatal development, or growth which continues until stopped by the inhibition produced by the excessive growth of the host; and the growth of which it is still capable (growth potential) after that of the host slows down or ceases. These two parts are in reciprocal relation to each other. The earlier the prenatal inhibition, the smaller and less differentiated will be the inhibited rest but the greater will be the remaining growth potential. Small embryonal rests may develop proliferative powers while large ones, which achieve a certain intrauterine development, seldom if ever show further capacity for spontaneous growth.

Trauma. The shift in the mode of the curve of carcinoma testis from twenty-three to twenty-nine years to thirty-five to forty-one years is due to an external stimulus.

In spite of the larger growth potential of the smaller testicular rests many do not achieve malignancy until their immediate region is traumatized. This is illustrated on comparing these tumors in the testicle and the ovary. A larger proportion of the latter are benign, forming adult structures. The chief difference in their histories is due to their locations; the testicle is exposed to injury, the ovary is not. It is generally admitted that teratomas do not become malignant much oftener than do normal tissues.

In the case of carcinoma testis we should expect with a history of injury to a small undifferentiated rest and the resulting proliferation of an embryonal cell, a shift in the mode of the curve towards that of old age cancer.

SUMMARY AND CONCLUSIONS

1. Growth potential and teratomas

1. Parasite. Teratomas are tridermal embryonal rests endowed with a certain amount of possible growth, i.e., growth potential. When the rest is comparatively large it has necessarily consumed considerable growth energy before birth, while in the small teratoma, the growth period may be divided into two parts, a slight early growth soon followed by an inhibition, and a later, or post-natal growth, should conditions permit.

2. Host. The total number of teratomas in a population, up to any given age, increases while growth potential of the hosts decreases. As the larger increases in the number of teratomas occur in periods when growth of the host is slowed most, the growth of the host must inhibit that of the embryonal rest.

3. The number of teratomas appearing in any given time varies inversely with the growth potential of the host and directly as that of the embryonal rest. The tumors which begin their postnatal growth before that of the host stops are of highest potential, but are not necessarily more malignant, for they must overcome a still present inhibition. Since most teratomas have a low growth potential, they appear most commonly at the time the growth of the host stops—from twenty-three to twenty-nine years.

II. Trauma and autonomous growths

1. Teratomas which start growth as a result of injury are malignant more frequently than those which proliferate solely under the influence of growth potential.

2. When trauma precipitates growth, the teratoma is frequently monodermal. If the inclusion is still in an undifferentiated condition the cell is often of an embryonal type. If a developed inclusion is traumatized the cell in many instances is like that of acquired cancer.

3. The curve of carcinoma testis rises and falls in a manner similar to that of teratoma testis and not like that of old age

cancer. This is another fact which may be adduced in support of the theory that carcinoma testis is a one-sided teratoma. In the female, a similar neoplasm might be expected to arise as the result of the physiologic stimulations of puberty. This is what actually takes place, hence the growth, in all probability, is of teratomatous origin.

4. Trauma is followed by proliferation of cells, and any precipitant of regeneration may be important in the etiology of acquired cancer. In the old the inhibition of the organism is almost negligible. Hence trauma at that time may readily be followed by an uncontrolled and therefore excessive growth. Thus, loss of growth restraint may be almost as important a factor in the etiology of acquired cancer as in that of congenital inclusions.

REFERENCES¹

- BARBON: Teratomata of the brain, *Jour. Cancer Res.*, 1916, i, 311.
CHRISTIAN: Dermoid cysts and teratomata of the anterior mediastinum, *Jour. Med. Res.*, 1902, vii, 54.
DESURMONT: Étude anatomo-clinique des tumeurs solides bilatérales des ovaires, Thèse de Paris, 1911-1912.
EWING: *Neoplastic Diseases*, Philadelphia, 1919.
EWING: Teratoma testis and its derivatives, *Surg., Gynec. & Obst.*, 1911, xii, 230.
LEXER: Ueber teratoide Geschwulste in der Bauchhöhle und deren Operation, *Arch. f. klin. Chir.*, 1900, lxi, 648.
NAKAYAMA: Ueber Congenitale Sacraltumoren, *Arch. f. Entwicklungsmechn. d. Organ.*, 1905, xix, 475.
STOCKARD: Developmental rate and structural expression, *Am. Jour. Anat.*, 1920-1921, xxviii, 115.
TARUFFI: *Storia della Teratologia*, 1881-1894.

¹ These references were chosen out of over one hundred and sixty; they contain extensive reviews of the literature consulted.

AN ATYPICAL ADENOMA OF THE PANCREAS ORIGINATING IN ISLET TISSUE

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True simple adenomata of the pancreas arising from the islands of Langerhans have been described by several authors. The condition is very rare and the diagnosis is somewhat difficult to establish. The tumors hitherto described have been very small, the largest being 11 mm. in diameter. Most of them were accidental autopsy findings, and some were discovered only in the sections prepared for routine microscopical examination. Nichols (10), in 1902, was one of the first to report such a tumor. He stated clearly and convincingly upon what grounds he based the diagnosis, and subsequent authors seem to have guided themselves according to those facts in deciding the islet origin of an adenoma of the pancreas as opposed to the other two possible sources, viz., duct and adult acini. Helmholtz (3) in 1907, Morse (8) in 1908, and Koch (6) in 1914, described cases which fulfilled the requirements stated by Nichols and concluded that they were dealing with a simple adenoma originating in the islands of Langerhans. LeComte (7) in 1913, described a case of his own and also reviewed the literature to that date. He determined that adenomata of the pancreas have no great clinical significance and "constitute no very serious hindrance to long life." He classified as true adenomata of the islands those reported by Nichols (10), Ssobolew (13), Reitmann (11), Herxheimer (4), Helmholtz (3), Morse (8), Cecil (1), Weichselbaum (14), Rollet (12), and Heiberg (2). Although he favors a diagnosis of adenoma in the above cases, some of the authors themselves, notably Ssobolew, Herxheimer, Weichselbaum, and

Cecil, either consider the nodules to be hypertrophic islands, or do not decide definitely between a diagnosis of simple hypertrophy and neoplasm. Whenever there is doubt between the diagnosis of simple hypertrophy and new growth he recommends the latter if the nodule exceeds the arbitrary diameter of 1 mm., provided, of course, that it shows all the characteristics of island tissue. Others have reported the same lesion and several have gone so far as to trace the origin of some carcinomata of the pancreas to the islands. The conclusions of some of these authors, especially of Fabozzi, who reported five cases, have been disputed vigorously. With the exception of Horgan (5) most of these investigators studied advanced carcinomata so that a doubt of validity of their conclusions seems justifiable.

The tumor of the pancreas to be described here was discovered at autopsy by Dr. H. T. Karsner, performed through the courtesy of Dr. John MacLachlan.

SUMMARY OF CLINICAL HISTORY AND PHYSICAL EXAMINATION

The patient is an unmarried white female fifty-five years of age, in whose past history nothing of importance is noted save that eight years ago a myomectomy was performed with uncomplicated recovery. Two years ago she noticed looseness of the bowels, which over several months progressively became worse and ultimately became a profound diarrhea. This was accompanied by gradual loss of flesh and strength but she was not confined to bed until about two months before death. In the last weeks of her life bowel movements were extremely frequent but well controlled, sometimes numbering 16 to 20 in the course of twenty-four hours. No microscopic or chemical examination of the stools was made but the gross examination showed thin watery stools with very little mucus and no blood. At various times indigestion of the different food elements was noted. On a milk diet the stools, upon standing, showed a supernatant layer of undigested fat. When meat was introduced into the diet, undigested fragments were seen in the stools. Fruits such as banana were easily recognizable. Fermentation was never marked nor was putrefaction severe. The clinical exami-

nation showed no abnormality of lungs, heart, abdomen, or nervous system. The patient finally died apparently as the result of profound asthenia.

SUMMARY OF AUTOPSY FINDINGS

The body is that of a white female apparently fifty-five years of age, showing moderate emaciation. Upon examination of the internal organs there were found, in addition to the tumor of the pancreas described in detail below, slight chronic interstitial nephritis, fatty metamorphosis of the liver, subacute enterocolitis, and slight passive congestion of lungs, liver, kidneys, and intestines.

SPECIAL EXAMINATION

Gross description

When the abdomen is opened the pancreas is found in the normal location, but a tumor mass partly embedded in the head presents above the lesser curvature of the stomach. This tumor is not adherent to any of the structures in the neighborhood. The pancreas measures 15 cm. in length. Partly embedded in the head of it, 10 cm. from the tip of the tail, is a mass, roughly spherical in shape and measuring 4.5 by 3.5 by 2.5 cm. This tumor is definitely and completely encapsulated so that it can be enucleated easily from the head of the pancreas. The capsule is thin, pearly white in color, and in it, coursing over the surface of the growth, are several dilated vessels, apparently veins. The tumor is firm but elastic and cuts with moderate resistance. The cut surface bulges moderately, is light yellow in color, and bleeds slightly. There is moderate vascularization of the tissue but no areas of hemorrhage are seen. Near the center of the mass there is one small area of necrosis about 3 mm. in diameter. The central portion of the growth is of a slightly deeper yellow color, is somewhat firmer, and bulges to a greater extent than the tissue at the periphery. The tissue is only moderately friable. The head of the pancreas is normal save for moderate congestion. The entire portion of the pancreas from the tumor to the tip of the tail is the seat of marked atrophy. In this

portion the cross section measures 12 by 5 mm. The lobules are obviously reduced in size, there is slight increase of the interlobular connective tissue, and a moderate amount of fat infiltration.

HISTOLOGICAL DESCRIPTION

Sections were stained by hematoxylin and eosin, eosin methylene blue, thionin, and Mallory's connective tissue stain.

Pancreas

The duodenal portion shows very advanced autolytic changes, so advanced, in fact that little description is justified beyond noting a slight increase in connective tissue both between and within the lobules. Several definite islets are found which are of normal size and structure. The tail shows great reduction in the size of the lobules, acini, and individual cells comprising them. There is a great increase of the interlobular as well as interacinar connective tissue, and in it are a few foci of lymphoid cells. The smaller ducts are well preserved and normal, but the larger ducts show desquamation of the lining epithelium in some instances and complete digestion in others. The islets of Langerhans are moderately abundant, of normal size and structure, and fairly well preserved. There is no sign of invasion of the pancreatic tissue by new growth or of neoplastic change in the cells of the pancreas.

Tumor

There is a definite but thin capsule which consists of dense, moderately nucleated fibrous connective tissue. This shows no sign of invasion by the parenchyma of the tumor. Throughout the tumor there are present anastomosing bands of connective tissue, some of which are densely fibrous and some rather loosely arranged. Many of these trabeculae do not join at all and are seen as isolated masses of connective tissue which vary considerably in size and in shape, some being elliptical or rounded, and others stellate or irregularly branching. Nearly every one of them contains one or more blood-vessels. Some of these

vessels are very thin-walled, consisting apparently of only a single layer of endothelial cells, while others are large and well formed with a thick wall consisting of intima, media, and adventitia. Most of the vessels are filled with blood. In some of the trabeculae there are seen merely blood spaces showing no definite endothelial lining. The trabeculae give to the parenchyma of the tumor an alveolated appearance. The parenchyma consists of numerous masses and anastomosing bands and strands of epithelial cells. These masses vary greatly in size and shape.

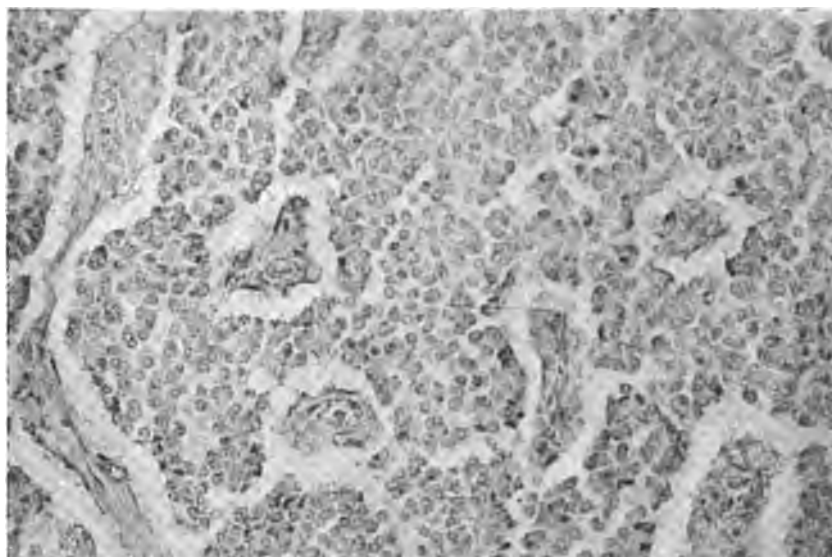


FIG. 1. PHOTOMICROGRAPH SHOWING TYPE OF CELL, ARRANGEMENT OF CELLS AND THE SUPPORTING CONNECTIVE TISSUE CONTAINING CAPILLARIES

Some are in intimate contact with the bands of connective tissue described above, but in most instances they are retracted from them. This is very likely due to fixation. Between the individual epithelial cells there is no reticulum. Cell outline in many areas is rather indistinct, but for the most part the cells are polyhedral or rounded in shape. Most of the cells are arranged in the form of solid masses, but some of the latter are hollowed out and in a few sections apparent attempts at acinar arrangement are seen, the cells surrounding these imperfectly

formed acini being of the cylindrical type. In many sections, what at first appear to be fairly well-formed acini are seen to contain in their lumen small masses of loosely arranged moderately nucleated connective tissue. These are formed apparently by the ensnaring of some of the connective tissue stroma by strands of epithelial cells which happen to be arranged in a circular manner. Many of these small areas of connective tissue within the pseudo-acini contain one or more small capillaries. Wherever the connective tissue is small in amount the epithelial cells come into such intimate contact with the capillaries that a spinous peritheliomatous appearance is the result. It is impossible to identify a well formed typical acinus, but in a few cases, owing to the cylindrical appearance of the cells and the pseudo-acinar arrangement one cannot rule out the possibility that this represents a feeble attempt at the formation of a tubule or gland follicle.

Most of the tumor cells are polyhedral or rounded in shape, are approximately of the size of islet cells, in most instances definitely smaller than those of pancreatic acini, and show the same variation in size that the islet cells do. An occasional very large cell is seen but this is not uncommon in the normal islet. The cytoplasm of the cells is relatively moderate in amount, is definitely acidophilic, stains very lightly, and is granular. As in the nuclei of the islet cells, the chromatin is aggregated into a single large mass or several, usually three or four, small clumps. The examination of very many sections failed to reveal a single mitotic figure.

The general architecture of the tumor as described above bears a very striking resemblance to that of the islets of Langerhans, so much so that if certain portions in which the masses and cords of epithelial cells are not large were seen in a normal pancreas they would fulfill all the morphological requirements of an island. A careful study of the cells themselves confirms this very striking resemblance.

SUMMARY

That this tumor is of benign nature is deduced from the following facts: It is definitely and completely encapsulated.

Neither the capsule nor the neighboring pancreatic tissue shows any signs of direct invasion by the new growth. There is no metastasis. There are no mitotic figures. The general arrangement of the tumor is not unlike that of some cellular adenomata found in other organs of the body. The epithelial cells of the tumor and their arrangement with relation to the vascularized trabeculae show unmistakable signs of differentiation, since there is such a striking resemblance to the islets of Langerhans. In size, shape, staining characteristics, and the minute structure of the cytoplasm and nucleus the cells resemble very closely those of the islands.

The tumor here described is in all respects like those reported by Nichols, Helmholtz, Morse, and others. The only differences are that this growth is much larger than any hitherto reported, and that by reason of its size and location it had exerted pressure upon the pancreatic ducts and had caused clinical signs and symptoms suggestive of pancreatic insufficiency. In this respect it differed from most adenomata hitherto described. Glycosuria was never found.

CONCLUSION

An adenoma of the head of the pancreas has been described which originated in an islet of Langerhans.

REFERENCES

- (1) CECIL, R. L.: *Jour. Exper. M.*, 1911, xiii, 595.
- (2) HEIBERG, K. A.: *Centralbl. f. allg. Path. u. path. Anat.*, 1911, xxii, 532.
- (3) HELMHOLTZ, H. F.: *Bull. Johns Hopkins Hosp.*, 1907, xviii, 185.
- (4) HERXHEIMER, G.: *Virchows Arch. f. path. Anat.*, 1906, clxxxiii, 228.
- (5) HORGAN, E. J.: *Jour. Lab. and Clin. M.*, 1920, v, 429.
- (6) KOCH, K.: *Virchows Arch. f. path. Anat.*, 1914, ccxvi, 25.
- (7) LECOMTE, R. M.: *Jour. Med. Res.*, 1913, xxix, 251.
- (8) MORSE, M. E.: *Jour. Amer. Med. Assoc.*, 1908, li, 1075.
- (9) MUSSEY, R. D.: *Med. Clin. North America*, 1919, iii, 681.
- (10) NICHOLS, H. G.: *Jour. Med. Res.*, 1902, viii, 385.
- (11) REITMANN, K.: *Ztschr. f. Heilk.*, 1905, xxvi, Abt. f. path. Anat., 1.
- (12) ROLLET, H.: *Frankfurter Ztschr. f. Path.*, 1912, x, 268.
- (13) SSOBOLEW, L. W.: *Virchows Arch. f. path. Anat.*, 1904, clxxvii, Suppl. 123.
- (14) WEICHSELBAUM, A.: *Sitzungsbericht d. kaiserliche Akademie d. Wissenschaft. in Wien, Mathem. natur. Klasse*, 1910, cxix, 73.

THE INFLUENCE UPON THE GROWTH OF TRANS-PLANTED FLEXNER-JOBLING RAT CARCINOMA OF HYDROGEN IONS AND OF VARIOUS SALTS IN DIFFERENT CONCENTRATIONS

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1. INTRODUCTION

In connection with the investigation of certain enzyme actions of extracts of malignant human and rat tumors, the results of which are published elsewhere (1), a study was made of the effects upon their subsequent growth of various treatments of the Flexner-Jobling rat carcinoma transplants before inoculation. The results presented here include the effects of solutions of different hydrogen ion concentrations and of some simple salts and their mixtures.

2. EXPERIMENTAL METHODS AND RESULTS

a. Tumor transplantation method

Albino rats, both male and female, were used in this work. They were inoculated with the Flexner-Jobling rat carcinoma in the usual way, and the tumors were allowed to grow for a period of from six to seven weeks (2). The term $\frac{\text{FRC}}{86\text{A}}$ for example, indicates that the tumor was derived from the 86th generation, series A. The rats were fed on wheat bread soaked in whole milk, fresh cabbage or carrots, and fresh tap water *ad libitum*.

In carrying out the transplantations, six (or more) small particles of tumor tissue each weighing about 5 mgm., and one piece weighing about 50 mgm., were selected from the non-

necrotic area of the rat tumor and placed in 25-cc. portions of solutions of definite hydrogen ion concentration and salt content previously sterilized in a steam autoclave at fifteen pounds pressure for fifteen minutes. The flasks containing the solutions and tumor fragments were allowed to remain in the ice box for definite intervals of time of from one-half to seventy-two hours. At the end of this period of time, the smaller pieces of tumor tissue were inoculated with a trochar into rats subcutaneously in the region of the right axilla. At the same time, the larger as well as some of the smaller pieces of tumor tissue were fixed in formaldehyde solution for histological examination. As a control, the same number of animals were inoculated with untreated tumor tissue immediately after its removal from the tumor-bearing animal. The progress of the transplanted tumors in the rats was recorded graphically by measuring them every week by means of calipers.

b. Preparation of solutions

The pH values of the solutions were determined in most cases by means of indicators and the standard solutions recommended by Clark (3), and in some potentiometrically. The salts used were purified by crystallization in the usual manner whenever necessary. The compositions of the various solutions in which the tumor fragments were immersed are given in table 1.

c. Growth of transplanted tumors after different treatments

The complete results of the transplantation experiments are recorded in 39 charts. As it is manifestly impracticable, even if desirable, to reproduce this number of charts, the results will be given in the first instance in table 1 as far as possible. Although this method of presentation leaves much to be desired, especially in the recording of the detailed growths of the various transplants, it will serve to bring out the points which are to be emphasized. In order to present a partial idea, at any rate, of the various growths, a number of more or less typical examples were chosen from the charts and are recorded in figure 1.

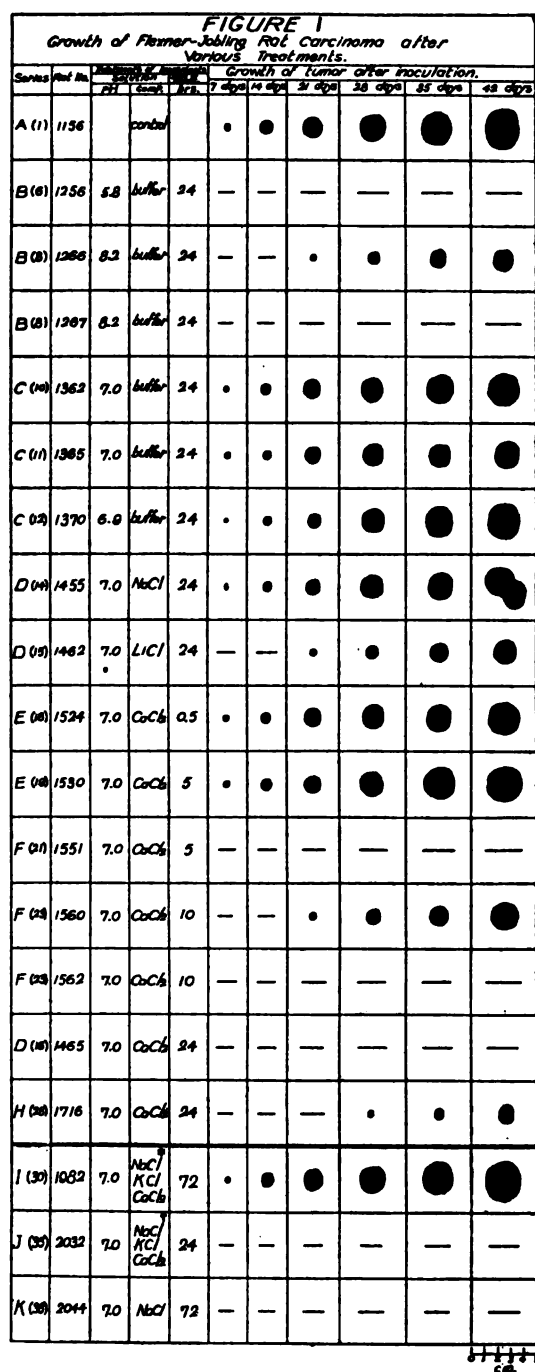


Fig. 1. Growth of transplanted Flexner-Jobling rat carcinoma after immersion in various salt solutions and in solutions of different hydrogen ion concentrations.

* Concentration of salts as in Locke-Ringer solution.

† Concentrations of salts three times those in Locke-Ringer solution.

TABLE 1
Results of transplanting Flazner-Jobling rat carcinoma after various treatments

SERIES	NUM- BER OF ANI- MALS USED	TUMOR USED	IMMERSION OF TUMOR FRAGMENTS BEFORE INOCULATION			GROWTH OF TRANS- PLANTS	REMARKS
			Composition of solutions	pH	Time of im- mer- sion		
A(1)	5	FRC 90B	Controls		hours	100	Rapid growth
A(2)	5	FRC 90B	50 cc. M/5 KH_2PO_4 + 1 cc. M/5 KOH, diluted to 200 cc.	5.1	24	0	No growth
A(3)	5	FRC 90B	50 cc. M/5 KH_2PO_4 + 29.6 cc. M/5 KOH, dil. to 200 cc.	7.0	24	75	One rat died shortly after the inocula- tion, one failed to develop the tumor, others grew normally
A(4)	5	FRC 90B	50 cc. M/5 KH_2PO_4 + 53.0 cc. M/5 KOH, dil. to 200 cc.	8.8	24	0	No growth
B(5)	5	FRC 91A	Controls			100	Rapid growth
B(6)	5	FRC 91A	50 cc. M/5 KH_2PO_4 + 5.7 cc. M/5 KOH, dil. to 200 cc.	5.8	24	0	No growth
B(7)	5	FRC 91A	50 cc. M/5 KH_2PO_4 + 29.6 cc. M/5 KOH, dil. to 200 cc.	7.0	24	(100)	With two rats, no growth in first week, normal thereafter; other two, normal growth at once; one rat, growth after second week

B(8)	5	FRC 91A	50 cc. M /5 KH ₂ PO ₄ + 51.0 cc. M /5 KOH, dil. to 200 cc.	8.2	24	(60)	With three rats, no growth for two weeks, then normal growth; with two rats, no growth at all
C(9)	5	FRC 91C	Controls			80	Four grew rapidly, one retrogressed after second week and disappeared in fifth week
C(10)	5	FRC 91C	35 cc. M /5 KH ₂ PO ₄ + 20.8 cc. M /5 KOH, dil. to 200 cc.	7.0	24	100	One tumor developed slowly, rest grew rapidly
C(11)	5	FRC 91C	50 cc. M /5 KH ₂ PO ₄ + 29.6 cc. M /5 KOH, dil. to 200 cc.	7.0	24	100	With two, no growth in first week, normal thereafter; other three, normal growth at once
C(12)	5	FRC 91C	65 cc. M /5 KH ₂ PO ₄ + 39.0 cc. M /5 KOH, dil. to 200 cc.	6.9	24	100	With two, no growth in first week, normal thereafter; other three, normal growth at once
D(13)	5	FRC 92A	Controls			100	Four grew rapidly, one began to retrogress after fourth week
D(14)	5	FRC 92A	0.15 M NaCl (0.88 per cent)	7.0	24	100	Slow growth during first week, rapid thereafter
D(15)	5	FRC 92A	0.15 M LiCl (0.64 per cent)	7.0	24	60	Two rats, no tumor growth; two rats, normal growth; one normal after second week

TABLE 1—Continued

SERIES	NUM- BER OF ANI- MALS USED	TUMOR USED	IMMERSION OF TUMOR FRAGMENTS BEFORE INOCULATION			GROWTH OF TRANS- PLANTS	REMARKS
			Composition of solutions	pH	Time of immer- sion hours	per cent	
D(16)	5	FRC 92A	0.078 M CaCl ₂	7.0	24	0	No growth
E(17)	5	FRC 94A	Controls			100	Rapid growth
E(18)	5	FRC 94A	0.078 M CaCl ₂	7.0	4	100	Rapid growth
E(19)	5	FRC 94A	0.078 M CaCl ₂	7.0	5	80	Four grew normally; one, no growth
F(20)	5	FRC 94B	Controls			100	Rapid growth
F(21)	5	FRC 94B	0.078 M CaCl ₂	7.0	5	20	One, normal growth; four, no growth
F(22)	5	FRC 94B	50 cc. M/5 KH ₂ PO ₄ + 5.7 cc. M/5 KOH, dil. to 200 cc.	5.8	5	100	Normal growth
F(23)	5	FRC 94B	0.078 M CaCl ₂	7.0	10	(40)	Three, no growth; one, normal growth after second week; one, normal growth after third week

F(24)	5	FRC 94B	0.078 M CaCl ₂	7.0	24	(80)	One, no growth; two, normal growth after second week; two, normal growth after third week
G(25)	5	FRC 96A	Controls			80	Four, normal growth, one rat died
G(26)	8	FRC 96A	0.078 M CaCl ₂	7.0	24	0	No growth
H(27)	6	FRC 97A	Controls			83	Five, normal growth; one tumor retrogressed after second week
H(28)	10	FRC 97A	0.078 M CaCl ₂	7.0	24	(70)	Three, no growth; four grew normally after second week; three grew normally after third week
I(29)	10	FRC 99B	Controls			100	One tumor grew slowly; rest rapidly
I(30)	5	FRC 99B	0.15 M NaCl 0.003 M KCl 0.003 M CaCl ₂	7.0	72	80	One tumor did not grow; others grew normally
I(31)	5	FRC 99B	0.45 M NaCl 0.009 M KCl 0.009 M CaCl ₂	7.0	72	0	No growth
I(32)	5	FRC 99B	0.45 M NaCl	7.0	72	0	No growth

TABLE 1—*Continued*

SERIES	NUM- BER OF AN- IMALS USED	TUMOR USED	IMMERSION OF TUMOR FRAGMENTS BEFORE INOCULATION				GROWTH OF TRANS- PLANTS	REMARKS
			Composition of solutions	pH	Time of im- mer- sion	per cent		
I (33)	5	FRC 99B	0.009 M CaCl ₂	7.0	72	0	No growth	
J (34)	10	FRC 100B	Controls			80	One tumor retrogressed; one did not grow; rest grew normally	
J (35)	5	FRC 100B	0.45 M NaCl 0.009 M KCl 0.009 M CaCl ₂	7.0	24	0	No growth	
J (36)	5	FRC 100B	0.009 M CaCl ₂	7.0	24	0	No growth	
K (37)	14	FRC 100C	Controls			93	One tumor retrogressed; rest grew normally	
K (38)	5	FRC 100C	0.15 M NaCl	7.0	72	0	No growth	
K (39)	5	FRC 100C	0.003 M CaCl ₂	7.0	72	0	No growth	

The data given in table 1 are perhaps sufficiently self-explanatory. The experiments were allowed to proceed six to seven weeks in every case. In the column showing percentage of positive inoculations, whenever the growth was delayed for a period greater than one week, it was calculated as a positive inoculation, but the number indicating the percentage was enclosed in parentheses. Figure 1 shows the relative growths under the different conditions more clearly.

The results of the various treatments on the growth of the transplants can be summarized in a comparatively brief manner as follows:

1. After immersion in potassium phosphate buffer mixtures of various hydrogen ion concentration for twenty-four hours, the following results were obtained: No effect was observable at pH 7.0, the growths being practically the same as in the control; no growths at all were obtained at pH 5.8 and 5.1; at pH 8.2 partial inhibition and delayed growths were found; and no growths at pH 8.8.

2. Varying the concentration of the buffer mixture (from approximately 0.6 per cent to 1.1 per cent) at pH 7.0 (series C10, C11, and C12) and immersing for twenty-four hours did not produce any effect, the growths being normal in all the cases.

3. Sodium chloride (0.15 M or 0.9 per cent) at pH 7.0 had no effect in twenty-four hours on the growth of the transplants; seventy-two hours immersion resulted in complete inhibition of the growths; lithium chloride at pH 7.0 in twenty-four hours caused partial inhibition.

4. Calcium chloride at pH 7.0 inhibited or retarded the growths of the transplants. Immersion for one-half hour had no effect; for five and ten hours, partial inhibition or retardation; for twenty-four hours and longer, complete inhibition.

5. Immersion in a Locke-Ringer solution for seventy-two hours did not affect the growths. Immersion in a solution containing three times the concentration of the sodium, calcium, and potassium chlorides in the Locke-Ringer solution for twenty-four or seventy-two hours resulted in complete inhibition of the tumor transplants.

d. Histological examinations of tumors

The histological examinations of a number of the tumor transplants after immersion in the various solutions were made by Dr. James Ewing, to whom the writers wish to express their thanks. The general structure after immersion in the phosphate mixtures of various hydrogen ion concentrations showed slight hydropic degeneration in the case of pH 7.0, but with the more acid and more alkaline solutions the tumor cells had undergone marked hydropic degeneration, being larger and considerably swollen. The microscopical structure of this tumor is shown in plates 1 and 2; in plate 1 an untreated section, and in plate 2 one treated with a solution of pH 5.8. The histological comparison of untreated tumor and tissue which had been immersed in solutions of sodium chloride, lithium chloride, and calcium chloride also showed some interesting facts. In those treated with sodium or lithium chloride, there was considerable accumulation of fluid between the tumor cells and many nuclei appeared slightly shrunken. On the other hand, tissue which had been treated with calcium chloride showed imbibition of fluid in the intercellular spaces and the cells themselves were shrunken. The nuclei stained more densely and the chromatin was condensed to a central mass within the nuclei. The cytoplasm showed hydropic vacuolization (plate 3). A microscopical examination of tumor tissues was made after immersion for seventy-two hours in a Locke-Ringer solution, and also of the same tumor tissue treated by a modified Locke-Ringer solution in which the salt content was increased three times. Under both conditions the tissues showed marked hydropic degeneration, but the proliferating capacity of the tissue treated with normal Locke-Ringer solution was not altered; while under the second condition proliferation was completely inhibited.

3. DISCUSSION

In comparing the results of the tumor transplanting experiments, it is necessary to consider not only the contents of the solutions in which the tumor fragments were immersed before

the inoculations, but also the lengths of time of the immersions. The temperatures were kept fairly constant, otherwise these also would have to be included. It will be seen that in discussing the contents of the solution, not only must the salt and its concentration be considered, but that the hydrogen ion concentration is of the greatest significance.

The favorable effect of immersion for twenty-four hours in solutions of pH 7.0 as compared with the more acid or more alkaline solutions is striking. Immersion in the pH 8.0 solution appeared to be less harmful than immersion in the pH 6.0 solution. Complete inhibition of growth was caused by the pH 6.0 and pH 9.0 solutions in twenty-four hours. From a histological examination of the tumor cells after immersion in solutions more acid or more alkaline than pH 7.0, it was found that there was a hydropic degeneration of the cells similar to that of tumor cells resulting from exposure to radium or to *x*-rays.

The action of the salts on the tumor tissue before inoculation is of interest. Sodium chloride had no effect in twenty-four hours, but exerted a harmful action in seventy-two hours. Lithium chloride produced a small inhibiting action in the twenty-four hour treatment. Calcium chloride caused a very strong inhibiting or retarding action on the subsequent growths even in ten hours. The use of potassium salts in the buffer mixtures showed that potassium exerted no specific retarding influence.

Cramer (4) several years ago studied the effects of sodium chloride and calcium chloride solutions on the growth of a transplantable mouse carcinoma. He allowed isosmotic solutions of these salts to act on the cells for one to two hours before inoculation. Marked inhibition of growth was shown by the cells immersed in the calcium chloride solution, practically none by those immersed in the sodium chloride solution. He found that the harmful effects of the calcium chloride could be overcome by subsequent immersion in sodium chloride solution, and that the action of the calcium chloride was only transient.

Although the experiments of Cramer are of interest in connection with the present work in showing a parallelism between

the harmful action of the calcium salt and the less harmful action of the sodium salt, the following differences in the manipulations, aside from the use of mice in the one case and rats in the other, may be pointed out. In the present work, the hydrogen ion concentrations of the solutions in which the tumor fragments were immersed were controlled; in Cramer's work they do not appear to have been controlled. In the former, the inoculated animals were observed for six to seven weeks after inoculation; in the latter for two weeks. In the former, also, the times of immersion were varied for a number of the treatments, and certain salt mixtures were used. In spite of these differences, the results of Cramer offer valuable contributory evidence in connection with the transplantation phenomena.

The results of the transplantation experiments described in this paper are evidently due to cell destruction of some form. In view of the harmful action of the salts separately, and the harmlessness of the "balanced" mixture, the action on the cell membrane or wall appears to be the dominating phenomenon. Destruction of the membrane or cell wall, or perhaps better, modification of its permeability, destroys the reproductive power of the cell. The actions of small amounts of acids and of bases, and of various salts and mixtures of these, have been described in connection with other cell structures a number of times. In the present work, the effects of solutions of different hydrogen ion concentrations have been made more definite, and the relative action of sodium and calcium salts at a definite hydrogen ion concentration brought out clearly. The explanation of these actions is, however, the same as that developed in other connections by a number of workers.

The work on the protease actions of malignant human and rat tumor extracts presented elsewhere (1) shows some interesting similarities and differences as compared with the transplantation phenomena presented here. While it is true that the causes underlying the two sets of phenomena are probably of entirely different nature, the protease results will be outlined briefly in comparison with the transplantation results.

The optimum hydrogen ion concentration for protease action and the favorable medium in which tumor fragments on immersion retain their ability to grow when transplanted, correspond very closely to that represented by pH 7.0, unfavorable conditions for both being reached more rapidly on the acid side than on the alkaline side. Chlorides of the alkalies in fairly dilute solution and within short time limits, did not affect appreciably the protease action or the ability of tumors to grow when transplanted, but calcium salts under similar conditions exerted a very marked inhibiting or retarding action on both. On the other hand, a definite difference is observed with a mixture of sodium chloride, calcium chloride, and potassium chloride in the concentration found in Locke-Ringer's solution. Retardation of the protease action was caused to the same extent that the salts separately would cause such action, while with the transplantations, the salts exerted an antagonistic action toward each other in the sense that no inhibition of the tumor growth was observable in the subsequent inoculations.

The two sets of actions, therefore, can be ascribed to different causes; the transplantation results in the first instance to salt actions on the permeability of the cell membranes, the enzyme results to chemical actions of unknown nature on the enzyme molecule or enzyme grouping of some molecule. The comparative actions of the sodium and calcium salts parallel each other when used alone, but not when used in mixtures in certain proportions and concentrations. The parallelism, even if due to different causes, as is probable, is of interest.

Certain facts may be mentioned in this connection, even if they have no direct connection with the phenomena described in this paper. In reviewing past work on the inorganic constituents of neoplastic tissue, it may be stated that the calcium content of rapidly growing tumors was found to be small. Calculating the potassium-calcium ratio of such tumors, large concentrations of potassium as compared with the calcium were found, while in old, necrotic, or slowly growing tumors, the concentration of calcium was considerably greater in comparison with that of the potassium (5).

Finally, it may be stated that while a definite connection may exist between the conditions affecting the permeability of cell membranes, the factors which influence the activities of intracellular enzymes, and the relations of the inorganic constituents of neoplastic or other tissue, such a connection is still obscure and can only be hinted at until more definite experimental evidence is available.

4. SUMMARY

The growth of the Flexner-Jobling rat carcinoma was investigated after grafts had been immersed in solutions of various salts of different hydrogen ion concentration. Calcium strongly inhibits growth, and a pH of 6.0 appears to be more harmful than pH 8.0.

The growth of the tumors was compared with the protease action of extracts of malignant human and rat tumors, and similarities and differences in these phenomena indicated, as well as possible explanations for them.

REFERENCES

- (1) J. Biol. Chem., 1922, liii, 75.
- (2) SUGIURA, K., AND BENEDICT, S. R.: Jour. Cancer Res., 1920, v, 373.
- (3) CLARK, W. M.: The Determination of Hydrogen Ions, 1920.
- (4) CRAMER, W.: Biochem. Jour. 1918, xii, 210.
- (5) BEEBE, S. P.: Am. Jour. Physiol., 1904, xii, 167.
CLOWES, G. H. A., AND FRISBIE, W. S.: Amer. Jour. Physiol., 1905, xiv, 173.
WATERMAN, N.: Arch. Neerlnd, Physiol., 1921, v, 305.

PLATE 1

The plates show three photomicrographs taken with the same magnification. Plate 1 shows untreated tumor tissue of the Flexner-Jobling rat carcinoma.

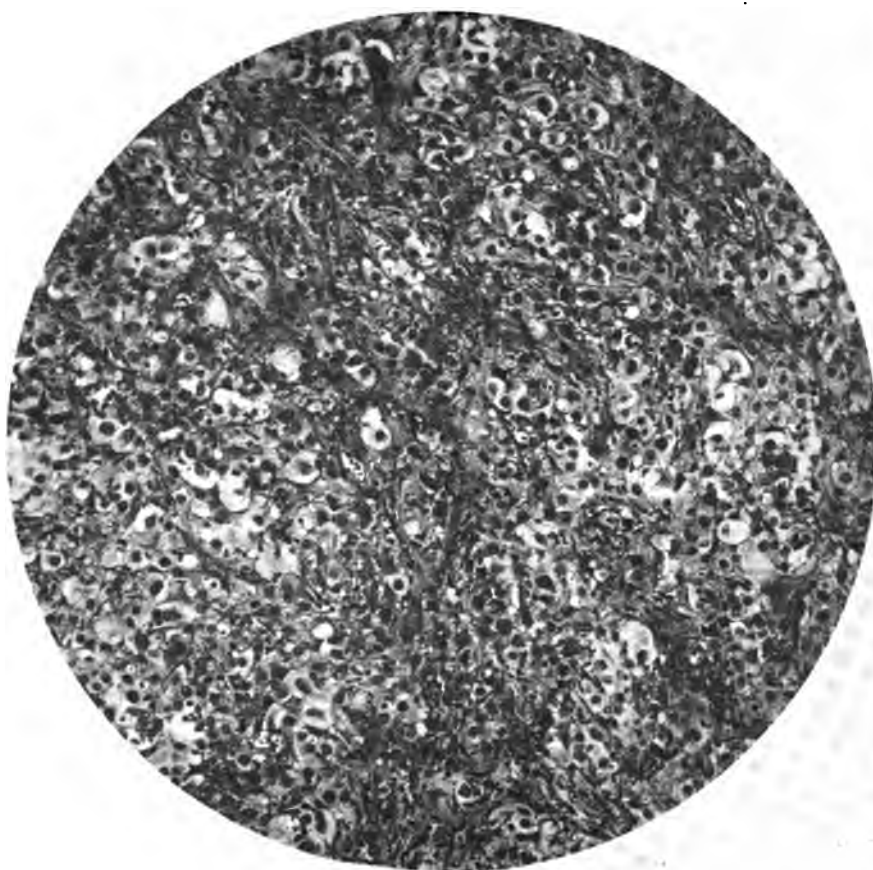


PLATE 2

Plate 2 represents the same tumor after immersion for twenty-four hours in the phosphate mixture, having the pH 5.8.

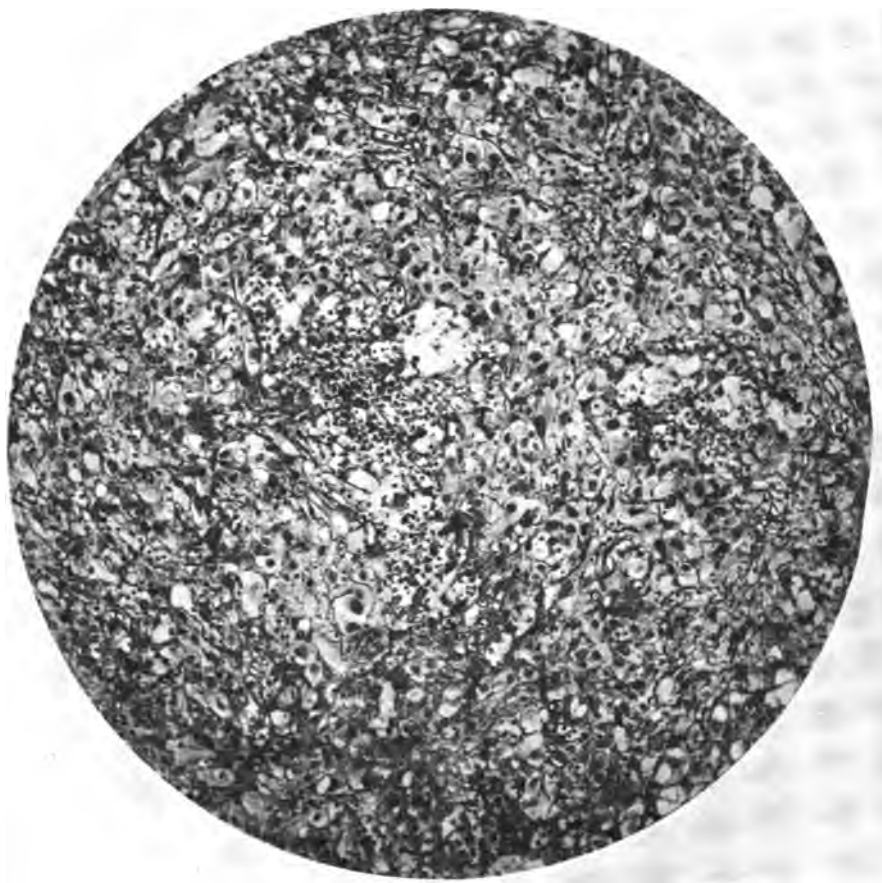


PLATE 3

Plate 3 shows the results after immersion of the same tumor for twenty-four hours in the 0.078 M calcium chloride solution, having the pH 7.0. Microscopical examination of the treated tissues showed considerable degenerative changes. (For further reference see text.)



PRIMARY SPONTANEOUS TUMORS IN THE KIDNEY AND ADRENAL OF MICE

STUDIES ON THE INCIDENCE AND INHERITABILITY OF SPONTANEOUS TUMORS IN MICE

SEVENTEENTH COMMUNICATION

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Primary tumors of the kidney occur not infrequently throughout the animal kingdom, and, in general, seem to exhibit the variations and peculiarities seen in human renal tumors. As an indication of the comparative pathology of renal tumors the following review of the literature is presented:

Mouse: Few cases of primary renal tumors have been described among the great numbers of other tumors found in this species. Tyzzer (1) found 4 renal growths, which he interprets as hypernephromas, among 83 primary tumors in mice. One of these mice, an old female, had also an adenoma of the lung, a carcinoma of the ovary, and a lymphoma infiltrating both kidneys; another had an adenoma in the lung. At the time this paper was written the interpretation of hypernephroma was more liberal than at present, and the illustrations might now be interpreted by many pathologists as papillary adenoma of the kidney, which was Tyzzer's original diagnosis in two of his cases.

Among the 300 mice with spontaneous tumors described by Haaland (2), there were but two renal tumors. One was a large growth in the kidney of a twenty-two months old male mouse, and had invaded a vein but produced no metastases; microscopically the structure was that of an adenocarcinoma. The second tumor, in a mouse of the same sex and age, resembled

perfectly in structure a human hypernephroma; there were no metastases, although the lung contained an adenoma. Inoculation of the hypernephroma into 40 mice was without result.

Murray (3) also described a spindle-cell growth surrounding the kidney without infiltrating it, apparently a primary retroperitoneal sarcoma and related to the kidney by position only.

Further than the above we can find no reports of primary renal tumors in mice, although Stumpf (4) has contributed a discussion of the behavior of carcinomas inoculated into the kidney.

Rats: Of 103 tumors found in 100,000 rats autopsied in plague work by McCoy (5), 11 were in the kidney, and were classified as 4 adenomas, 6 carcinomas, and 1 papilloma. Woolley and Wherry (6) in 23,000 rats found 22 tumors, of which 3 were in the kidney; all were of renal-cell type. Bullock and Rohdenburg (7), in a compilation of the literature on rat tumors, found 123 (including those cited above) of which 7 were fibroadenomas, 1 a papilloma, and 8 carcinomas of the kidney. Among 32 cases of their own in laboratory white rats, there were 10 adenomas of the kidney. Therefore, of 155 rat tumors 26 were of renal origin. A further case of carcinoma of the kidney in a wild rat was reported by Beatti (8). Loewenstein (9) described tumor-like papillary outgrowths arising in the pelvis of the kidney, as well as in the ureter and bladder, of rats infected with *Trichodes crassicauda specifica*.

Since sarcomas are much more common than carcinomas in rats, the fact that all the renal tumors in rats are of epithelial structure is of interest.

Squirrels: In 250,000 ground squirrels (*Citellus beecheyi*) autopsied in plague work, McCoy (10) found 8 tumors, of which one was described as an angiosarcoma of the kidney. A case of hypernephroma in the kidney of a grey squirrel (*Sciurus carolinensis pennsylvanicus*) was reported by Fox (11).

Birds: Fowls are rarely subject to renal tumors. Of 880 examined by Curtis (12), 79 had tumors, 5 of which were in the kidneys; but these were not microscopically corroborated and there is reason to doubt their true neoplastic nature. In 34 cases of tumor in fowls compiled by Wernicke (13), not one was

primary in the kidney although in 3 cases renal metastases were found. In 852 autopsied fowls Bürger (14) found 12 tumors of which none was in the kidney, although a sarcoma of the ovary had produced metastases in the kidney in one case.

The review of the literature on tumors in fowls and birds by Joest and Ernesti (15) who collected 112 cases and added about 50 more, reports no further renal tumors.

Other birds seem to have renal tumors perhaps more frequently, as is indicated by the following reports: White (16) described a fibrosarcoma in the kidney of a goose. Fox (17) reported in the kidney of a male chestnut eared finch (*Amadina castanotis*) a medullary carcinoma, becoming scirrhus in places, with metastases to the lungs, and three cases of benign adenoma of the kidneys in undulated grass parakeets (*Melopsittacus undulatus*). He had previously reported (18) two cases of papillary cystadenoma and also a "cyst adeno-carcinoma papilliferum" (19) in the same species; besides an adenocarcinoma of the kidney in a saffron finch (*Sycalis flaveola*), (20) and a spindle-cell sarcoma of the left kidney with metastasis into the left tibia in a scaly ground dove (*Scardapella squamosa*) (21). Seligmann (22) reported as a diffuse carcinoma a growth which involved both kidneys, with metastases in the liver and mesentery, in a Chilean pintail (*Dafila spinicauda*) which was twenty-six years old. Baird (23) reported a case of keratinizing epithelioma in the kidney of a fowl, and Borrel and Masson (24) also described a renal tumor in a fowl which showed both cylindrical and squamous elements.

Domesticated mammals. Rabbits, which are less subject to tumor than most mammals, seem to have a relatively large proportion in the kidney, for Scott (25) stated that of 39 new growths reported, 5 were benign renal adenomas, resembling Wilms' tumors in structure.

Swine also seem to be particularly subject to renal tumors, for of the 12 cases of tumors in swine collected by Sticker, 7 arose in the kidney, and other cases reported since then indicate the same tendency. Many of these tumors were of the mixed embryonal tumor type.

In horses renal tumors are far from rare. In Sticker's (26) compilation, of 509 cases of malignant tumors in horses 37 were in the kidneys. McFadyean (27), in 63 cases of tumor in animals included 5 in the horse kidney, none with metastases. Of 142 equine neoplasms among 77,224 horses slaughtered in Japan, there were 9 in the kidney, as compared with 49 in the testicle (Kimura) (28).

According to Trotter's (29) figures, renal tumors are much less frequent in cattle, for of 305 cases but 1, a colloid cancer, arose in the kidney; but Sticker's figures show 10 of 78 bovine tumors in the kidneys. Steinke (30) described as hypernephroma a tumor of the kidney in a cow. Roussy and Wolf (31) in their review on cancer in animals, gave a picture of a tumor from a bovine kidney resembling a hypernephroma, and stated that cancer of the kidney in horses resembles that in man, while in swine the adenocarcinoma of the kidney is among the most common tumors. Cadiot (32) quoted a case of an enormous cancer of the kidney in a mare.

No reports of cases of renal tumors in sheep can be found.

Of 766 primary cancers in dogs in Sticker's tables 19 were in the kidney. McFadyean also described two renal tumors in dogs, each case exhibiting metastasis. No renal tumors appeared in the 21 cats with tumors in Sticker's lists, nor in Roffo's 7 cases (33) nor in Murray's 11 cases (34) but Teutschlaender mentioned the cat (Kater) on his list of animals in which carcinoma of the kidney has been reported. Murray also described 48 cases of tumors in dogs, 12 in horses, and 18 in cows, with none arising in the kidneys.

We are indebted to Dr. L. E. Day for his summary of 316 tumors found among 2000 animal specimens sent to the Chicago Laboratory of the Bureau of Animal Industry. These do not represent all the tumors that are observed in the slaughter house, but merely specimens sent to the laboratory by the inspectors when in doubt concerning the diagnosis. Among 175 tumors of cattle there were no renal tumors although there were four adrenal tumors, two diagnosed as sarcomas and two as hypernephromas. Among 93 tumors in pigs no less than 52 were in the kidney,

47 being diagnosed as embryonal adenosarcomas and 5 as sarcomas; there were no adrenal tumors. In considering the tumors in swine it is to be remembered that nearly all swine are slaughtered before they are two years old, so that not many of the slaughtered animals have reached an age for developing carcinomas. Among 48 tumors from sheep none whatever were found in the kidney or adrenal. These figures emphasize the infrequency of renal tumors in cattle and sheep and the frequency of mixed tumors of the kidney in swine, which resemble the typical mixed tumors of the human kidney.

Other incidental cases are: Sarcoma of the kidney in a swine (Hamburger) (35), the report of which leaves some uncertainty whether the growth may not have been leukemic.

Papillary adenoma in the left kidney in a mongoos lemur (White) (36). A tumor involving the kidneys, liver, lungs, and ovaries of a python, the primary site not being determined (Bland-Sutton) (37). A leiomyoma in the kidney of a horse, described by Wells (38). Pick (39) described a soft adenoma involving both kidneys of an eel. Williams (40) stated "A malignant renal tumor (derived from an adrenal 'rest') has been described by Bland-Sutton in a marmot." In Teutschlaender's compilation (44) were listed the following cases from the literature: Hemangioma of the kidney in a horse; carcinoma of the kidney in fowl, frog, pike, and buzzard.

While the above compilation is certainly not entirely complete, it serves to bring together much of the literature on the comparative pathology of renal tumors, and to indicate their general distribution and frequency.

Adrenal tumors. We can find no reports of primary adrenal tumors in mice, despite the great numbers of mice that have been examined at autopsy and the not infrequent occurrence of adrenal tumors in other species. Thus, Kimura (42) found in the literature records of adrenal tumors in 24 horses and 46 cattle, and he himself found 5 adrenal tumors among 46 tumors from horses. The extensive discussion of the comparative pathology of adrenal tumors by Steinke (43) mentioned tumors only in horses and cows, commenting on the absence of such tumors in

other species except for an adrenal tumor of parasympathetic-cell character in a two-year-old swine, reported by Klawitter.

There is, however, the interesting case reported by Smallwood (44) as a carcinoma of the kidney of a frog and believed to be derived from the adrenal tissues. Murray, who reexamined this specimen, corroborated its carcinomatous nature, but his statement suggests that he was not convinced that it developed from adrenal rather than renal cells. The illustrations in Smallwood's article suggest a papillary renal tumor.

Fox (45) described as a hypernephroma a tumor that arose in the adrenal of a California hair seal (*Zalophus californianus*), and also described a hypernephroma in the adrenal of a brown cebus (*Cebus fatuellus*) (46).

As stated previously, in the 316 animal tumors examined by Day in the Chicago Stock Yards, but 4 arose in the adrenal, all in cattle, 2 being diagnosed as sarcoma and 2 as adenoma.

RENAL TUMORS IN MICE IN THE SLYE STOCK

In 33,000 autopsies performed on mice of the Slye stock, but 16 cases of unquestionable primary tumor arising in the kidney have been observed, supporting the impression that the kidney of the mouse is not among the common sites of primary neoplasm. These renal tumors are, according to the histological evidence, to be classified as follows: 1 carcinoma, 3 adenomas, 1 hypernephroma, 7 sarcomas, 3 mesotheliomas, and 1 sarcoma in the renal pelvis. It will be noted that we have observed no instances of the mixed tumors of the kidney that are so common in man and some other species, nor have we had any papillary tumors of the renal pelvis. The features presented by these several types of renal tumor are best described by giving briefly the findings in each of the several cases that we have observed.

EPITHELIAL TUMORS OF THE KIDNEY

1. Primary carcinoma of the kidney (fig. 1). Female mouse (1934). This mouse showed a white solid mass in the right kidney about the size of a normal kidney. Beyond this there were no

abnormalities found, except that the spleen was about three times the normal size. The left kidney was normal, and no metastases were found. Microscopically the tumor is composed of cells of epithelial type, but without the characteristics of either adrenal cells or of tubular epithelium of the kidney. They are arranged

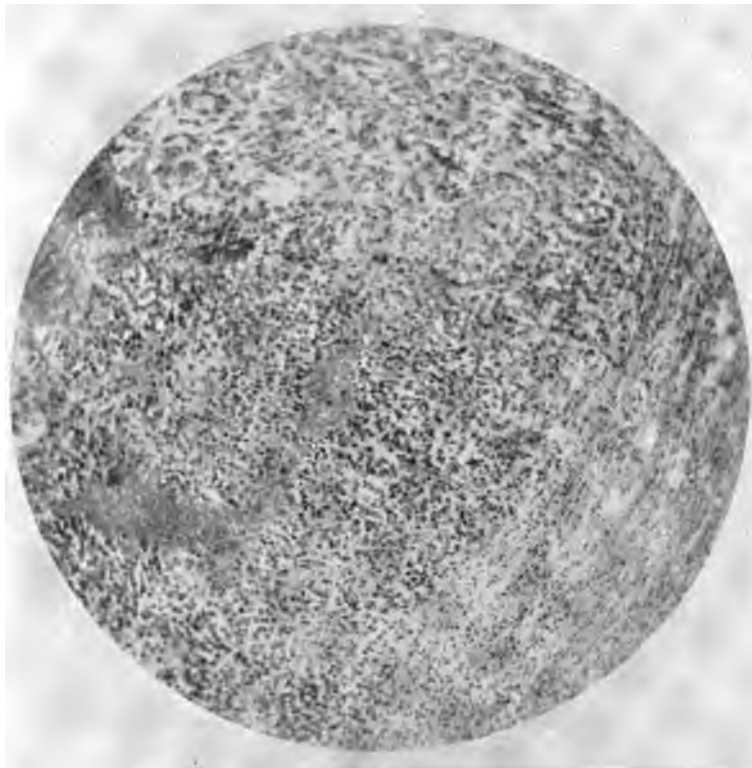


FIG. 1. PRIMARY CARCINOMA OF THE KIDNEY

The junction of the compressed renal tissue and the tumor is shown. Apparently this tumor is derived from renal epithelium. Mouse 1934. $\times 110$.

in large, pseudo-alveoli with much central necrosis, so that the persistence of the tumor cells near the bands of stroma gives in places the impression of a papilloma. Although the growth does not infiltrate the kidney very much, it has no capsule of its own, infiltrates the renal capsule in places, and is undoubtedly

malignant. Evidently it is a tumor derived from the renal epithelium and may be properly designated as a carcinoma, although the term mesothelioma might also be appropriately used. Presumably it is in an early stage of malignancy in view of the relatively small amount of extension of the growth.

2. Solid adenoma arising in bilateral cystic kidneys. Male mouse (9907), age two years, thirteen days. Both kidneys were converted into masses of small cysts of various sizes, resembling the congenital cystic kidneys, although no cysts were found in the liver. Some of the cysts contain colloid masses, and between them are occasional foci of small round cells. There remains more kidney tissue in a functional condition than is usually seen in fatal human cases of congenital cystic kidney, but this tissue is far from normal, the tubules containing many hyaline casts, the interstitial tissue being infiltrated with round cells, and many of the glomeruli being more or less hyalinized. In the lower pole of each kidney was a fleshy nodule about 3 to 4 mm. in diameter. Microscopically these nodules are composed of a solid tissue, made up of groups of large epithelial cells somewhat resembling renal epithelium. About these is a delicate stroma containing some collections of small round cells. The nodule is distinctly encapsulated and seems to be in the renal substance rather than within one of the cysts. In many respects the structure resembles that of the benign ovarian adenomas of mice (47). No abnormalities of importance were noted in the other organs.

3. Adenoma of the kidney (fig. 2). Male mouse (10220), which died with advanced sarcosporidiosis; had marked chronic nephritis, both kidneys being large and nodular, with atrophic areas alternating with areas of swollen and dilated tubules, some of the latter being distinctly cystic. Beneath the capsule of one kidney was a nodule 4 mm. in diameter, with a well defined capsule. It consists of a mass of large epithelial cells with solid cytoplasm, arranged in large cords with a very delicate stroma between them. The arrangement of the cells resembles that of the adrenal adenomas, and a diagnosis of benign hypernephroma might readily be made. The cells are less vacuolated than those

of the typical adrenal cortex, having a denser cytoplasm, and are not dissimilar to the epithelial cells found lining the distended renal tubules; it seems probable, therefore, that this adenoma is derived from renal epithelium. There are some clefts containing

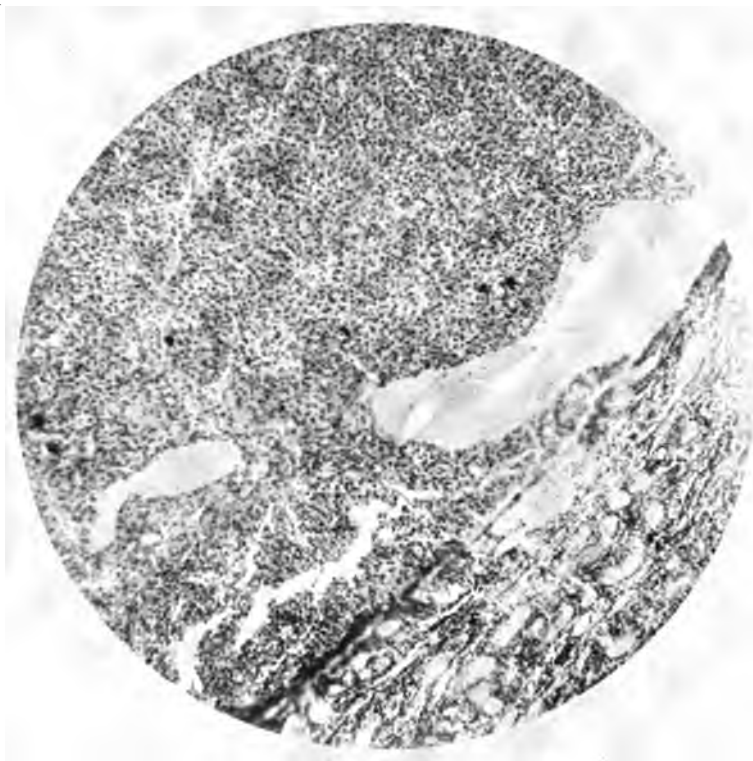


FIG. 2. ADENOMA OF KIDNEY

This somewhat resembles a tumor derived from adrenal cells, but is believed to have been derived from renal epithelium. Mouse 10220. $\times 65$.

colloid material, and numerous calcific granules in the form of calcospherites.

4. Adenoma of the kidney. Male mouse (24073), with a large liver cyst containing a tapeworm, exhibiting in one kidney a solid subcapsular nodule 5 mm. in diameter, with a delicate but definite capsule. This is composed of a solid mass of large

epithelial cells with abundant foamy cytoplasm and small dark nuclei. The cells are in large cords or alveoli with a very delicate stroma between them. There are numerous small foci of calcification of the tumor tissue. The foamy character of the tumor cells, together with their arrangement, suggests an adrenal

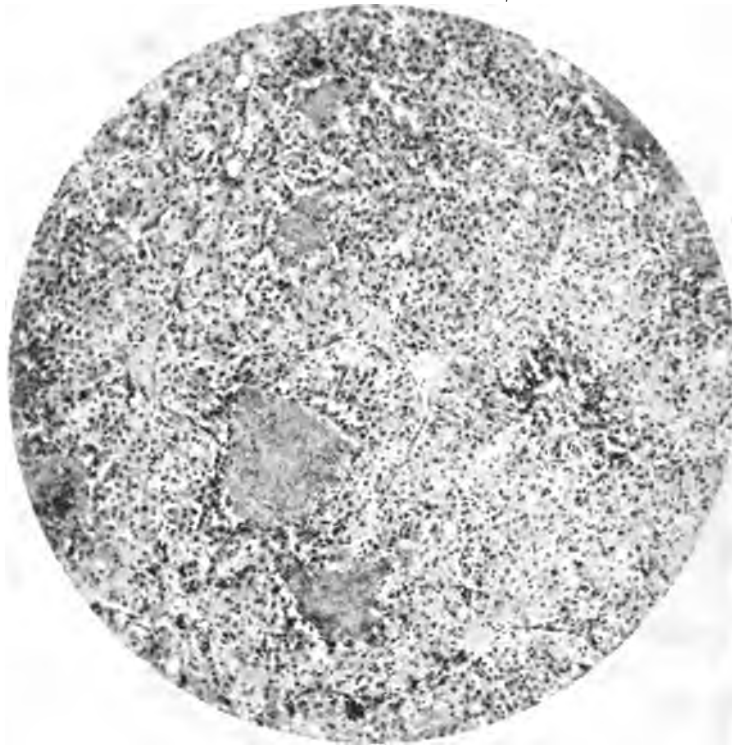


FIG. 3. RENAL HYPERNEPHROMA

This growth entirely replaced one kidney, but produced no metastases; it corresponds in structure to human hypernephromas. Mouse 3639. $\times 110$.

origin for this growth, but the post-mortem changes have so altered the nuclear and cytoplasmic details that this cannot be determined positively. In places there is a tendency to tubular arrangement of the tumor cells, suggesting a renal origin. Outside the tumor the kidney shows an advanced chronic nephritis with many hyaline and fibroid glomeruli.

5. Hypernephroma in the kidney (fig. 3). Male mouse (3639) with no other lesions of significance. The left kidney was replaced by an encapsulated, hemorrhagic mass, measuring 22 x 22 x 18 mm. The right kidney was slightly enlarged and soft, and showed a moderate degree of chronic nephritis. No remains of renal tissue are found in the tumor mass, which presents the typical appearance of a hypernephroma. More than half the mass contains no cells, being composed of the residue of old hemorrhages and necrosis. The living portions consist of large foamy cells arranged in cords and alveoli with a delicate stroma. It is completely surrounded by a capsule which shows no invasion by tumor cells. In all respects this growth corresponds perfectly to the human renal hypernephromas. No metastases can be found.

In the hilum of the right kidney, attached to a large artery, is a nodule about 2 mm. in diameter which, in the center, resembles a small leiomyoma, but about it is a mass of granulation tissue. It bears no resemblance to the hypernephroma and its nature is unknown.

In view of the fact that chronic nephritis is one of the commonest diseases in mice, and appears in forms often quite similar to nephritis in man, it is strange that we have found so few instances of epithelial neoplasms in mice, especially the benign adenomas which are so often found in human kidneys showing chronic nephritis.

SARCOMA OF THE KIDNEY

The diagnosis of sarcoma, always difficult and often unsafe, is particularly dubious in the case of the kidney which presents so many non-sarcomatous growths that resemble sarcoma, and especially in the face of the statement made by Ewing (48): "Birch-Hirschfeld's group of adeno-angiosarcoma, derived from Wolffian remnants, and the lipomyosarcomas remain, however, the only well-defined varieties of renal sarcoma which have been fully divorced from a probable epithelial origin." Nevertheless, since the kidney contains connective tissue it is perfectly possible for sarcomas to arise therein, and in this mouse material we have

several specimens for which only the diagnosis of sarcoma can be made, after excluding all other possibilities. In doing this we have carefully eliminated numerous growths of doubtful character, some of which also may really be sarcomas.¹ These cases are briefly described as follows:

6. Bilateral sarcoma of kidneys. Male mouse (7667), with both kidneys symmetrically enlarged to equal size, about 17 x 10 x 9 mm. They contained little recognizable kidney tissue, and were for the most part infiltrated by a fleshy, pinkish white tissue which involved equally the cortical and medullary portions. No lesions were found elsewhere. Microscopically both kidneys show infiltration replacing about 80 to 90 per cent of the renal elements. The neoplasm is composed everywhere of slightly oval cells, a little larger than lymphocytes, with deeply staining nuclei and very little cytoplasm. No evidence of neoplastic epithelial or mixed tumor elements can be found. This tissue infiltrates between the tubules much as do the cells in leukemic infiltrations, and the capsule is also invaded. The diffuseness of this infiltrative growth and its equal involvement of both kidneys makes it resemble a leukemia or pseudoleukemia, but this diagnosis is untenable in view of the lack of involvement of other organs or lymph-nodes. The diagnosis of sarcoma is made largely by exclusion.

¹ We wish to quote here a statement of the criteria used in our consideration of sarcoma throughout this work as expressed in our paper on Primary Spontaneous Sarcoma in Mice (Jour. Cancer Res., 1917, ii, 1). "We recognize fully the difficulties that attend the differentiation of sarcoma, and for the purpose of this study have excluded every form of new growth concerning the nature of which there seemed any possible room for question. Therefore, we have not included numerous cases in which we think that the growths are probably sarcomatous, and many more in which we cannot be sure that the neoplasm is not sarcoma. On the other hand, the statistical value of our figures is lessened by the fact that we have undoubtedly omitted some growths that are true sarcomas. Our figures represent minimal values only. From the standpoint of investigations in heredity, with which our work is particularly concerned, it is just as undesirable to call a sarcoma something else as to include a granuloma among the sarcomas, and hence the rigid classification adopted in this study of sarcomas is no more satisfactory for our heredity statistics than would be a lax classification that included some growths of doubtful nature. Therefore, in charting the heredity statistics it is necessary to recognize the absence of positive criteria for the differentiation of sarcoma, and to admit the borderline cases with a mark of interrogation to indicate this fallibility."

7. Sarcoma of kidney. Old male mouse (24979), with the right kidney entirely replaced by a tumor, 16 x 12 x 10 mm., which infiltrated the adjacent tissues, including the ureter and the pelvis of the opposite kidney. The regional lymph-nodes were also invaded, and there was a mass in the root of the mesentery about 20 mm. in diameter, which seemed also to infiltrate the pancreas.

Microscopically, the right kidney is found to be almost completely replaced by a growth of large round cells, uniform in size, with very little cytoplasm, but with nuclei much larger than those of lymphocytes. There is no tendency to structural arrangement, the tumor cells infiltrating freely the tissues about the kidney, including the adjacent muscles and the pelvis of the opposite kidney, the ureter of which is surrounded by a mass of tumor. The regional lymph-nodes are replaced by tissue of the same character, but the mesenteric mass is completely necrotic, with few cells resembling those of the tumor. By virtue of its highly infiltrative character and the large size of the cells, this seems to be an undoubted sarcoma. The other tissues showed no evidence of either leukemia or pseudoleukemia.

8. Sarcoma of the kidney. Female mouse (26867) with much sarcosporidiosis, showed a marked enlargement of the left kidney, which was between two and three times the normal size, and infiltrated diffusely with a pink, fleshy tissue. Elsewhere in the body there were no important changes. Microscopically it is found that the enlarged kidney is infiltrated extensively, nearly all the renal elements being replaced by a tissue composed of round and elongated cells, considerably larger than lymphocytes and with more cytoplasm. They tend to be arranged in wide bands, growing out from the blood-vessels, but this arrangement is not constant or well developed. The neoplastic tissue grows out from the pelvis and tends to invade the adjacent tissues and the hilum of the opposite kidney. No similar tissue is to be found elsewhere in the mouse. The probable diagnosis is primary sarcoma of the kidney.

9. Sarcoma of the kidney. Female mouse (27148), with the right kidney much enlarged (18 x 15 x 32 mm.) and consisting of

a firm tissue overlaid with softer portions. Microscopically it is found that the kidney is diffusely infiltrated with a growth consisting of polyhedral cells, smaller than epithelial cells usually are, but with a little more cytoplasm than lymphoid cells. These cells exhibit no structural organization and have replaced all but a few of the original renal elements. About one-third of the tissue is necrotic. A small amount of the neoplastic tissue infiltrates the hilum of the left kidney, which also shows amyloidosis. No similar tissue is found elsewhere in the body, and there are no evidences of diffuse lymphoid hyperplasia or similar conditions. The lung contains a small benign adenoma.

The tumor shows much variation in the size of the nuclei, hyperchromatism being common, and occasional very large cells are seen. Unfortunately there is too much post-mortem change for exact details to be seen. The general character of the growth and the absence of similar changes in other tissues make a diagnosis of sarcoma seem inevitable.

10. Bilateral sarcoma of kidneys, with metastasis in spleen. Female mouse (396), with the uterus enormously distended with fluid because of vaginal obstruction by a seminal mass, and with great enlargement of both kidneys by a uniform white tissue. The right kidney measured 18 x 13 mm., the left 15 x 13 mm. On the anterior surface of the left kidney is a depressed scar. In the spleen there is a tumor nodule, 4 x 5 mm. No other tumor growths were found. The adrenals were normal.

Microscopically, both kidneys are found to be infiltrated diffusely, with replacement of 80 to 90 per cent of the renal tissue by a growth uniformly composed of small oval cells in solid masses, without any particular organization. No similar tissue can be found in any other organ except the spleen, which has a tumor nodule of the same structure as the renal tumor. The adrenals are in contact with, but not invaded by tumor. The tumor forms large masses in the hilum of one of the kidneys, or within the kidney itself. The left kidney is more involved than the right, and probably was the starting point of the sarcoma.

11. Lymphosarcoma of kidney. Female mouse (13124), with two separate mammary carcinomas, and in the left kidney a

nearly spherical mass about 14 mm. in diameter, which was partly necrotic. Microscopically this growth is composed of a dense mass of small round cells without visible cytoplasm, packed closely together without attempt at formation of any structure. It infiltrates the adjacent renal tissue freely, and there is a small amount of infiltration of the hilum of the opposite kidney. The structure is of distinctly neoplastic character, and there is no similar infiltration of other organs, or lymph-node enlargement, such as characterizes pseudoleukemia. The diagnosis of sarcoma, in structure corresponding to lymphosarcoma, is the only one that can be made on the features presented.

12. Lymphosarcoma of kidney. This seems to be similar to case 11, but unfortunately post-mortem decomposition has advanced so far that accurate microscopic study is not possible. Such tissue as remains stainable resembles a lymphosarcoma. The mouse was a female (12533) with no other tumors, and the right kidney was replaced by a soft pink mass measuring 14 x 12 x 12 mm. A small amount of the same tissue invaded the hilum of the left kidney.

MESOTHELIOMA OF THE KIDNEY

In our previous papers on tumors in the ovary (49) and testicle (50) of mice we have called attention to the not infrequent occurrence of tumors composed of polyhedral cells, presenting some characteristics resembling carcinoma and some resembling sarcoma, (figs. 4, 6, 7 and 8) and hence suitably designated mesothelioma, in accordance with Adami. These tumors are characteristic of the urogenital anlage, and hence it is not surprising that growths of the same structure are found in the adrenal and kidney. We have observed the following cases of renal tumors that seem to belong to this group.

13. This is a remarkable case in that a female mouse (21663), when *but one month old* exhibited two independent carcinomas of the mammary gland, and osteosarcomatous growths in the spinal column near the pelvis and in the left fifth rib. It lived but eighteen days more and at autopsy showed its left kidney also almost completely replaced by a tumor measuring 18 x 12 x

10 mm.; the right kidney, similarly involved, measured 10 x 6 x 6 mm. (fig. 4). These renal growths are entirely different in structure from the other four tumors. They are alike and vary in appearance in different parts, some portions resembling

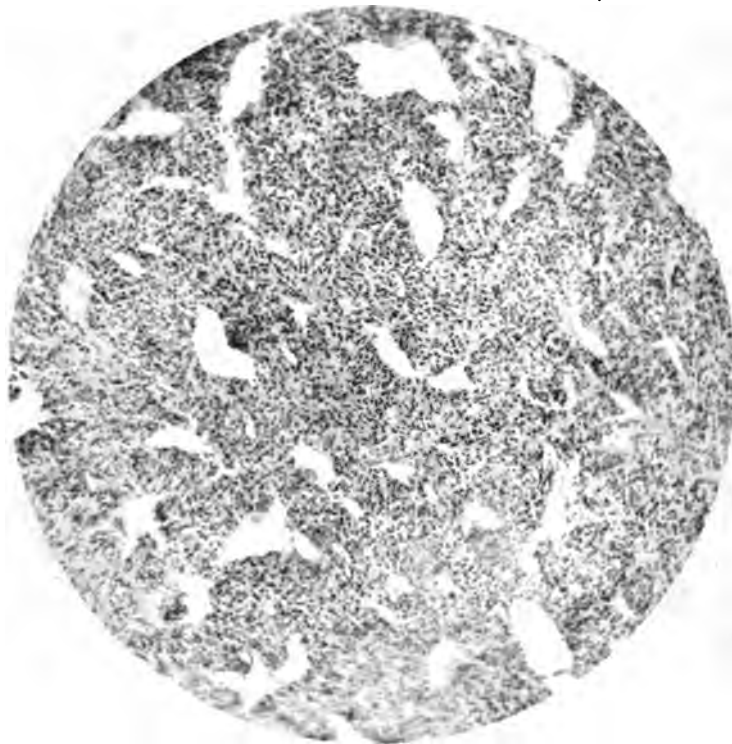


FIG. 4. MESOTHELIOMA OF KIDNEY

This portion of the growth presents a sarcomatous character; other portions exhibit more resemblance to epithelial growth. The tumor was found in a mouse but one month old, with four other tumors, which if not congenital must have developed very soon after birth. Mouse 21663. $\times 110$.

spindle-cell sarcoma while others are composed of larger, more polyhedral cells, arranged in a somewhat alveolar fashion, often separated by highly vascular septa. This structure corresponds to the type of growth often seen in tumors of the ovaries, testicles,

and adrenals in mice, and agrees with the tumors called mesothelioma by Adami and Woolley. Since, except for this case, there have been very few other cases of malignant tumors arising in mice less than six months of age, the occurrence in so young an animal of at least four independent primary growths representing three distinct types of malignant neoplasm, is a most remarkable condition, without, as far as we know, a parallel in either mouse cancers or in those of any other animal.

14. Male mouse (10011), with no other lesions of importance, had its right kidney largely replaced by a whitish tumor, forming a mass measuring 14 x 12 x 10 mm. Microscopically the tumor has largely replaced the kidney and has infiltrated the capsule in places. It is composed of small cells with a dark, round nucleus and a small amount of cytoplasm. These cells tend to form bands or pseudo-alveoli in some places, but for the most part the growth is composed of cells in a structureless mass. The left kidney was not involved by the tumor.

15. This seems to be similar to case 14. Male mouse (9779) had an enlarged left kidney without other lesions of note. Microscopically the kidney is largely replaced by a mass composed of small cells slightly larger than lymphocytes and with more cytoplasm, showing no structural arrangement. The main neoplastic mass lies at one side of the kidney, which it infiltrates slightly. The kidney itself shows some foci of round-cell infiltration. Some of these areas slightly resemble the tumor, but probably are not a part of it. There are some small areas of calcification, some scars, and numerous hyaline casts. Post-mortem changes are too advanced for more accurate study. The opposite kidney contains no tumor, but there is the same amount of amyloid and calcification.

SARCOMA OF RENAL PELVIS

We have excluded numerous cases in which a retroperitoneal tumor of sarcomatous character has invaded the kidney hilum, but there is one case in which both the gross and microscopical findings distinctly indicate that the tumor had its origin in the tissues of the pelvis itself.

Female mouse (348) had a pale swelling extending downward and inward from the pelvis, composed of tissue of about the same consistency as the kidney and of uniform yellow color. On cross section the pelvis of the kidney was in the center of the mass formed by the tumor and the kidney, which measures 12 x 9 mm. The left kidney was of normal size with a pale area of swelling on the anterior surface. No other changes of importance were found except severe edema of the lungs.

Microscopically the growth is found to lie symmetrically about the pelvis of the kidney and the upper end of the ureter, invading the wall of these structures extensively. From here it passes along the vessels deeply into the kidney, but does not extend far into the cortex. The kidney is about one half as large as the tumor, the tubules being much dilated and the glomeruli more or less hyalinized. The tumor also extends some distance along the renal capsule as a thin layer. As no growths are found elsewhere it is evident that this growth arose in the tissues about the hilum of the right kidney. There is only a small amount of invasion of the left kidney hilum. The growth is composed of large round cells with a delicate reticulum. There are several delicate vessels packed with lymphoid cells, but no other evidences of lymphatic origin in this tumor. The cells of the tumor are polymorphous with considerable cytoplasm, and deeply staining nuclei much larger than those of the ordinary lymph-cell, and usually larger than the nuclei of the renal epithelium. No mitoses are seen. No changes of importance are found in the other organs.

This tumor is much less complex in structure than the cases of sarcoma of the renal pelvis in children described by de Vecchi and Salomon (51).

ADRENAL TUMORS

As mentioned above, no cases of adrenal tumors have hitherto been described in mice. We are able to report a few unquestionable instances of such tumors. Despite the frequency of benign cortical adenomas of the adrenal in man, we have found but one such tumor in mice. It may be recalled that adrenal tumors have not been found frequently in other species of animals.

Cortical adenoma of misplaced inter-renal adrenal rest (fig. 5). Between the kidneys of a female mouse (1921), with marked amyloidosis and chronic nephritis, was found a spherical mass about 5 mm. in diameter, attached neither to the kidneys nor to the intestines. Both adrenals were present at their normal site

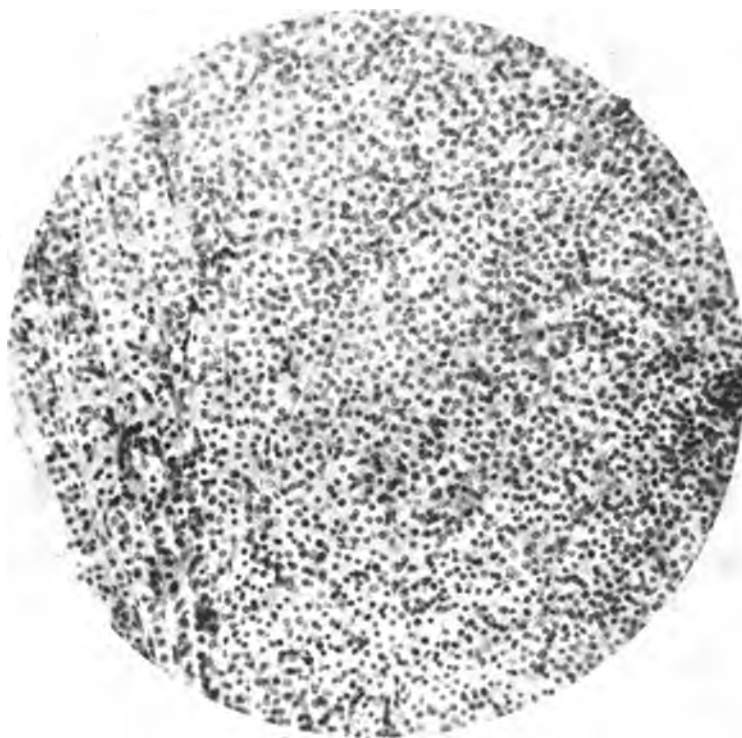


FIG. 5. CORTICAL ADRENAL ADENOMA

The tumor developed in an inter-renal rest of adrenal cortex tissue. A small zone of compressed adrenal tissue is seen at one side. Mouse 1921. $\times 225$.

and were of normal structure and size, except for some peripheral round-cell infiltration.

The tumor nodule is composed of a solid mass of cells resembling those of the adrenal cortex except in lack of orderly arrangement, closely packed together, and flattening out a thin shell of

adrenal cortex, evidently all that remains of an adrenal rest, since no medullary elements are to be found. In all respects this tumor corresponds to the simple adenoma of the adrenal cortex seen in man.

MESOTHELIOMA OF THE ADRENAL

This seems to be the commonest tumor of the adrenal, as also of the testicle, of mice, and it is quite impossible to distinguish on the basis of microscopic appearance between the mesotheliomas arising in the different organs derived from the urogenital anlage. The cases in which the diagnosis seems certain are the following:

1. Male mouse (10390) had in place of the right adrenal a spherical mass 5 mm. in diameter, which is completely encapsulated and does not involve the kidney. No other nodules or findings of importance elsewhere. Microscopically the nodule contains no remains of adrenal tissue, but consists of a solid tumor made up of masses of large cells with considerable cytoplasm and large oval or spherical nuclei, arranged in atypical alveoli or broad bands with a very small amount of stroma containing thin-walled blood-vessels. In numerous places the capsule is infiltrated with tumor cells and there is some invasion of the areolar tissues about the adrenal, but no invasion of the adjacent kidney. This seems to be a typical mesothelioma of the adrenal in an early stage of malignancy.

2. Mesothelioma of adrenal with peritonea¹ metastasis. Female mouse (12744), with abdomen greatly distended by a bloody exudate, presented at the site of the left adrenal a whitish mass about the size of the kidney. All through the abdominal cavity are masses of partially necrotic whitish tissue, especially attached to the liver and uterus, which do not seem to be involved by this growth. There are enlarged retroperitoneal lymph-nodes, but elsewhere no lymphatic involvement. There are no tumors in the lungs.

Microscopically the tumor shows the usual features of the typical mesotheliomas, as described in the other cases, with slight tendency to alveolar arrangement. It does not seem to have

infiltrated or produced metastases in the viscera. The right adrenal is surrounded, but not invaded, by the tumor and seems normal. The retroperitoneal nodes are replaced entirely by tumor tissue.

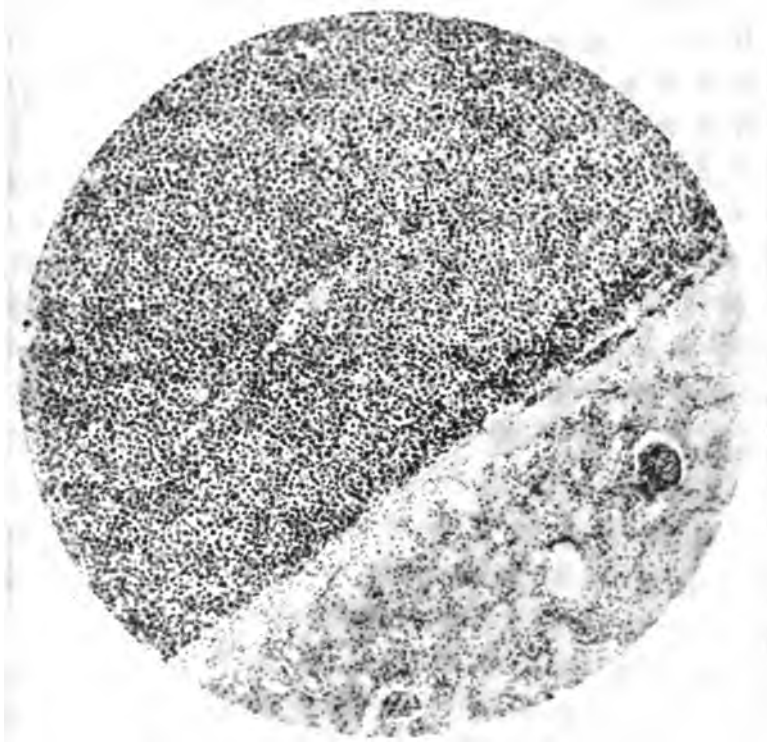


FIG. 6. MESOTHELIOMA OF ADRENAL

This growth surrounded the lymph nodes and was widely disseminated through the perirenal tissues. Mouse 7699. $\times 110$.

3. Bilateral malignant mesothelioma of adrenals (fig. 6). Female mouse (7699) with much subcutaneous edema, showed numerous enlarged subcutaneous lymph-nodes, some red and some pale, the largest measuring as much as 8 x 10 mm. There was a milky fluid in the peritoneal cavity, presumably because of pressure of enlarged lymph-nodes on the thoracic duct, for

the retroperitoneal nodes were greatly enlarged, up to 6 x 12 mm. The mesenteric nodes were also enormously enlarged, one mass at the root of the mesentery measuring 40 x 20 x 20 mm. Both adrenals were greatly enlarged, each being about the size of the kidney, which was adherent to the adrenal but not enlarged or infiltrated by neoplastic tissue. The uterus seemed to be infiltrated by tumor. The liver was not affected, and the lungs showed only a single small nodule, although there was some tissue increase in the upper mediastinum; both lungs showed a bloody edema, and there was a bilateral hydrothorax.

Although the gross appearance suggested a general lymphosarcomatosis or pseudoleukemia, yet the microscopic structure is of an entirely different character. Everywhere the neoplastic tissue presents the same appearance, being composed of a solid growth of cells with considerable cytoplasm, so that they look much like epithelium. The nuclei, which vary greatly in size, are much more solid than those of epithelial cells usually are. Mitotic figures are abundant. The invaded lymph-nodes are largely replaced by tumor cells, which are also found in the lymph-vessels of the lungs, but not in the liver. Both adrenals seem to be entirely replaced by the tumor, which lies upon the capsule of the adjacent kidney without any invasion of this organ. There are some areas of hemorrhage, but not much necrosis. The cells show no attempt at definite arrangement, but simply form a solid mass with numerous, poorly defined blood spaces.

This tumor resembles in structure other growths found arising in the urogenital anlage, and in view of this and of the complete replacement of both adrenals it is most probable that it did arise in these tissues, although the extensive lymph-node involvement is unusual in adrenal tumors. The structure is not essentially dissimilar to that of the adrenal tumor 10390, except for the amount of extension.

UROGENITAL MESOTHELIOMAS OF UNCERTAIN ORIGIN

Because of the identity in appearance of mesotheliomas from all organs originating in the urogenital anlage, it is not always

possible to decide the place of origin of some tumors which involve two or more of these organs, as shown by the following cases.

12307. Mesothelioma of either adrenal or ovary. It is not possible to determine the origin of this tumor, which we have described in our paper on tumors of the ovary in the following words:

The abdominal cavity shows several nodules whose exact origin is difficult to determine as the mate has partly devoured the body. The right ovary is, however, easily distinguished. It measures 18 x 12 x 12 mm. What seems to be the left ovary is 10 x 8 x 6 mm. There are 8 other similar nodules in the abdominal cavity, one being in the position of the left adrenal, measuring 10 x 8 x 8 mm. The other nodules are apparently in the mesentery. One lobe of the liver is converted into a tumor nodule 14 x 10 x 18 mm., irregular and lumpy in outline, pink in color.

The tumor shows everywhere the same structure, consisting of irregular alveoli composed of large cells with abundant cytoplasm with well defined borders and deeply staining nuclei. Mitotic figures are numerous. The character is that usual to mesothelial growths. The ovary cannot be positively identified, but one mass exhibited in the capsule structure suggests compressed ovarian tissue with degenerated ova. In all respects this tumor is identical with the malignant ovarian tumors just described.

It seems probable that this tumor arose in the ovary which exhibited the largest growth, but it is not possible to exclude the adrenal as the primary site.

The malignant tumors of the adrenal, ovary, and testicle commonly exhibit identically the same histological picture as that seen in this case.

We have also observed two other cases, described in the paper on ovarian tumors in which we cannot state whether the renal growth was primary or secondary.

12876. The left kidney contained a mass of pink, fleshy tissue, 18 x 14 x 14 mm. The right kidney, which was slightly enlarged, contained no tumor. The right ovary consisted of a pinkish tissue resembling that in the kidney, and measured

12 x 8 x 8 mm. In the mesentery was a similar, slightly paler mass, 16 x 8 x 8 mm. The retroperitoneal and subcutaneous nodes were not enlarged and no nodules were found in the lungs.

Microscopically the tumor is alike in all three places, consisting of a diffuse infiltrating growth of large round cells, which also invade the connective tissues about the kidney and ovary. It does not at all resemble the typical ovarian tumors, being apparently a round-cell sarcoma. We have no way of telling which of the three tumors was primary. The next case presents similar difficulties.

26. This mouse had a tumor mass about 8 x 10 mm. in the upper portion of the liver, with other smaller nodules near it. A similar small nodule was found in the right kidney. The right ovary was enlarged to two-thirds the size of a kidney, and was solid. Microscopically all these growths are composed of round cells, apparently a round-cell sarcoma. It is impossible to say which growth was primary.

Among several tumors arising in the retroperitoneal tissues, mostly of sarcomatous type, are a few of a structure identical with the characteristic mesothelioma type of growth that arises in the urogenital anlage. In the two cases described below this character of growth was so marked that it seems probable that the tumors have arisen in some misplaced embryonic rest, since the organs of this series were distinctly not the starting point of the growths. Numerous tumors of this sort have been described in man.

(22380) Malignant retroperitoneal mesothelioma with extension through the body wall. Male mouse presenting externally a subcutaneous tumor, involving the left hip and extending to the anus, the external measurements being 30 x 25 x 25 mm. When the body was opened it was found that this mass was an extension of a huge mass (40 x 20 x 18 mm.) which had surrounded the left kidney and pushed it to the ventral midline, invaded the spleen and pushed it mostly to the right of the midline by formation of a mass that measured 20 x 15 x 13 mm., and extended downward through the pelvic cavity where it surrounded the rectum and seminal vesicles and broke out through the

body wall. There was slight infiltration of the lower pole of the right kidney. The liver was slightly enlarged and mottled with areas of infiltration. There was some tumor in the lower part of the mediastinum, and the lungs show many small spots of neoplastic tissue. There seemed to be no involvement of the

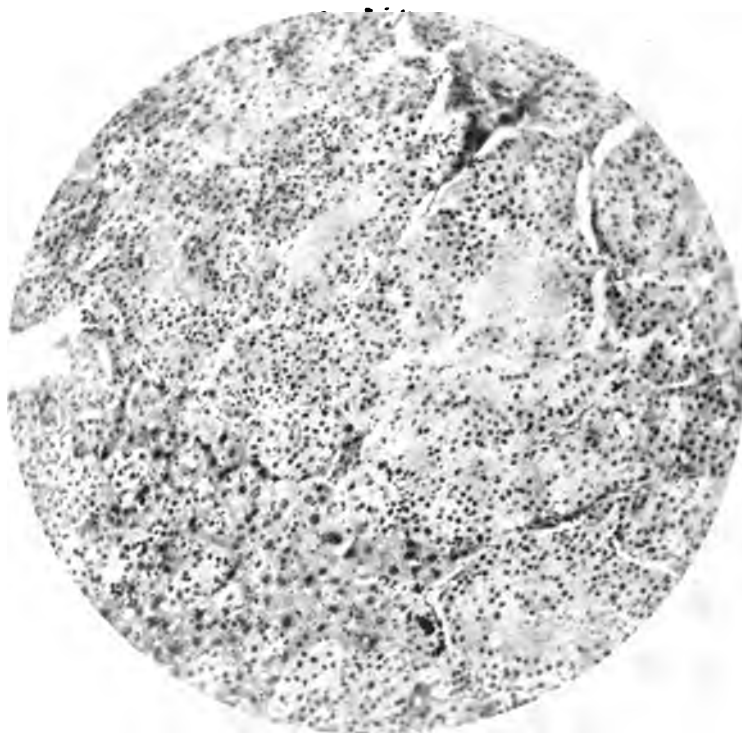


FIG. 7. SECONDARY MESOTHELIOMA IN LIVER

The primary growth was either in the adrenal or in a retroperitoneal embryonic rest; it infiltrated the body wall and retroperitoneal tissues, and produced innumerable metastases in the lungs and liver. This section shows the masses of tumor cells compressing and replacing the larger liver cells. Mouse 22380. $\times 110$.

lymph-nodes, either subcutaneous or abdominal. The testicles were not involved.

Microscopically the tumor everywhere consists of masses of large cells with much deeply staining cytoplasm, somewhat resembling liver cells, with a slight tendency to arrangement in

cords or bands. Where the tumor invades the liver it can be seen that the cells are a little smaller and paler than the liver cells (fig. 7).

The growth infiltrates the liver widely, invades the muscle of the body wall, invades the renal capsule but not the kidney,

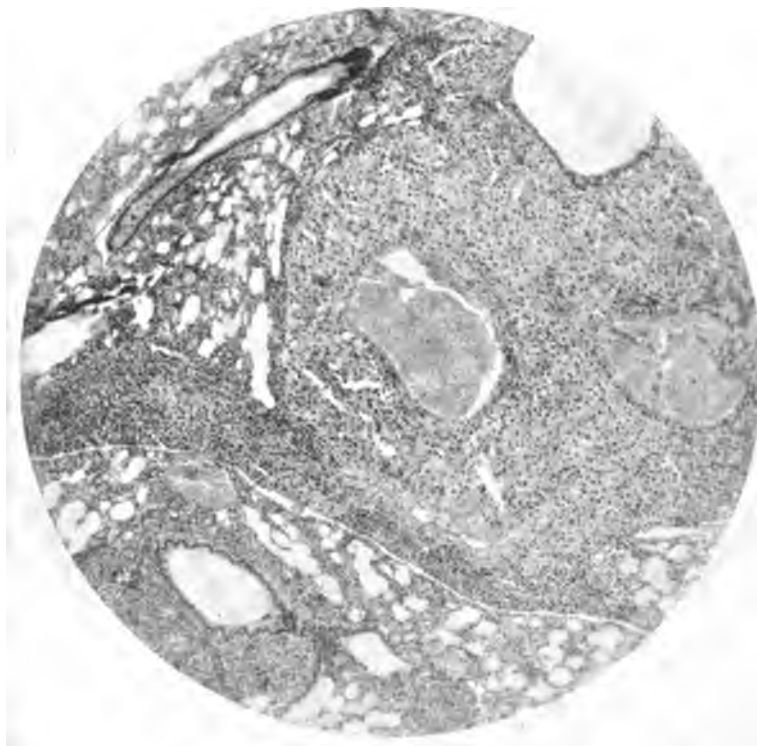


FIG. 8. SECONDARY MESOTHELIOMA OF LUNGS

From the same case as figure 7. The section shows the extent of involvement of the lung. Mouse 22380. $\times 60$.

infiltrates the abdominal sympathetic ganglia, surrounds densely the rectum and spreads into the seminal vesicle, invades the spleen, and in the lung appears as multiple tumor cell emboli within the vessels and as large tumor nodules largely replacing portions of the lung (fig. 8). The left adrenal was found entirely embedded in but not infiltrated by the tumor.

The microscopic appearances are identical with those usual in malignant adrenal tumors, but the adrenal was not involved; and in view of the retroperitoneal origin, the growth may be presumed to have arisen in an embryonal rest of the urogenital anlage.

(9979) Malignant retroperitoneal mesothelioma infiltrating kidneys. Small female mouse with extensive infiltration of the right thigh by a typical spindle-cell sarcoma, and with a mass above the left kidney, about as large as the kidney itself, which was infiltrated by the growth. The entire mass measured 20 x 14 x 10 mm. The right kidney was not so much enlarged. The growth seemed to have arisen at the site of the left adrenal, and to have pushed the kidney forward. The liver was invaded by tumor and enlarged. No metastases were found elsewhere. Microscopically the tumor about the kidney bears no resemblance to the spindle-cell sarcoma of the thigh, being composed of a mass of polyhedral cells arranged in solid masses, with a slight tendency to form bands or cords. The nuclei are not much larger than those of lymphocytes, but the cells have much more cytoplasm. The chief mass lies above and behind the left kidney, which shows considerable invasion through the capsule and about the blood vessels. There is a similar invasion of the right kidney but much less tumor about it. The right adrenal cannot be located; the left is free from tumor although there is a necrotic area between it and the kidney. The liver shows a large nodule of the same sort of tumor.

SECONDARY TUMORS OF THE KIDNEYS AND ADRENALS

The mouse kidneys seem to be extremely insusceptible to metastatic invasion by tumors. In our entire series of primary tumors of mice, now in the neighborhood of 5000 cases, of which the predominating form is carcinoma of the mammary gland, we have never met with a metastatic growth from one of these tumors into the kidneys, with one possible exception, despite the frequency of pulmonary metastases, which often replace most of the lung. The only metastatic carcinomas of the kidney that we have seen have been found

in four cases of primary carcinoma in the lung (3098, 11777, 12373, 14242). A photograph of the first of these four cases appears in our paper on lung tumor (52). These carcinomas of the lung are especially likely to produce extensive metastases, and the suggestion of some authors that these pulmonary growths in mice should not be included among the true tumors is evidently based on ignorance of their character. The fact that they have furnished our only instances of metastatic carcinoma in the kidney is sufficient evidence of their true neoplastic character and the high malignancy of some types.

Although we have seen many instances of widespread metastasis in sarcoma, the kidneys are almost immune from vascular sarcoma embolism. In our series of 87 cases of sarcoma in mice, although 23 showed metastasis but one hematogenous secondary nodule was found in the kidney, this coming from a mediastinal sarcoma (11791). Since then we have seen one other case of vascular metastasis of sarcoma into the kidney, from a sarcoma of the uterus (12058), with a large nodule of the same structure almost replacing the lower pole of the right kidney, and metastasis in the right ovary. Even the widespread growths of small round cells, which resemble the condition called lymphosarcomatosis in man, seem to affect the kidney but little, for among a considerable number of such cases we have but one with a distinct metastatic nodule in the kidney (7572), although several cases show extension from retroperitoneal metastases into the capsule, and then into the kidney. On the other hand, the kidneys exhibit extensive diffuse infiltration in leukemia and massive perivascular growths in pseudoleukemia in mice as in man.

The malignant retroperitoneal growths, most of which resemble lymphosarcoma, and the malignant tumors derived from the adrenals, commonly invade the kidney by direct extension into the hilum, often very extensively. The sarcomas primary in one kidney also tend to spread into the hilum of the opposite kidney. This ready invasion of the hilum of the kidney is a point of similarity of human and mouse neoplasms.

No instance of tumor metastasis into the adrenal has ever been observed, except possibly in the few cases of widespread mesotheliomatous growths invading both the ovaries and adrenals, the origin of which is uncertain.

SUMMARY

In a series of 33,000 autopsies on mice of the Slye stock, dying natural deaths at all ages, but as far as possible living out their natural span of life, there have been observed the following cases of true primary neoplasm arising from renal or adrenal tissues: First, from the kidney, 16 tumors, classified as follows: 1 carcinoma, 3 adenomas, 1 hypernephroma, 7 sarcomas, 3 mesotheliomas, and 1 sarcoma of the renal pelvis. Second, from the adrenal, 4 tumors, as follows: 1 cortical adenoma from a misplaced inter-renal adrenal rest, 3 mesothelial tumors. Third, five cases of tumors of the mesothelial structure characteristic of urogenital anlage neoplasms, but of which the exact origin could not be determined because of their widespread growth at the time of death. As these 25 tumors occurred in 33,000 mice presenting not far from 5000 other tumors, they are evidently uncommon tumors of mice, at least in this particular stock.

It will be noted that in this series there has been no instance of a mixed renal tumor of the Wilms type, which is so common a type of renal tumor in man and apparently also in swine. Although inflammatory conditions are very prevalent in the kidneys of mice, epithelial tumors are rare, and especially to be noted is the absence of even a single case of typical malignant hypernephroma, although one benign growth of this type was found. No epithelial tumors of the renal pelvis were found, although there was one case of sarcoma that seemed to take its origin in the pelvis.

Several instances of malignant retroperitoneal tumors have been observed, mostly of sarcomatous structure, which usually invade the kidney. These have not been included in this series, except two cases in which the structure resembled that of the

mesotheliomas, suggesting that the tumor had its origin in misplaced rests of the urogenital anlage.

Secondary tumors have never been found in the adrenals, and but rarely in the kidneys. Although this series includes at least 3000 cases of mammary carcinoma, often with widespread metastases in the lungs, we have never seen a secondary carcinomatous growth in the kidney. The only secondary carcinomas of the kidney as yet observed are four cases in which the primary carcinoma was in the lung, thus establishing the true neoplastic nature of these lung growths. In but two cases have metastatic sarcomas been seen in the kidney, if we exclude the numerous cases of invasion of the kidney by direct extension from para-renal growths.

As to sex: In the entire group of renal and adrenal tumors, we have equal numbers in males and females, agreeing with the observation made on other tumors in mice that, in tumors not peculiar to the sex glands, there is usually little difference in the incidence in the two sexes.

Differing from the tumors previously studied, coincidence of other tumors with the renal and adrenal tumors is uncommon. One mouse in this series had a spindle-cell sarcoma of the thigh. One mouse had a small, benign papillary adenoma of the lung. Only two mice had a mammary carcinoma, and one of these (21663) was a remarkable case, for this animal, when but one month old, was found with two independent mammary carcinomas, and with osteosarcomas in the spinal column and in a rib. It lived eighteen days more, and at autopsy there was also found a mesotheliomatous type of growth involving both kidneys. Except for this unique case there have been practically no instances of malignant tumors in mice less than four months of age, and few under six months. Most of the renal sarcomas occurred between the ages of seven months and one year, which is somewhat earlier than the usual time of appearance of epithelial growths; this, of course, corresponds to experience with human neoplasms.

The epithelial renal and adrenal tumors furnished no illustration of metastasis, but in three cases of sarcomatous or meso-

theliomatous growths there was noted involvement of the adjacent lymph-nodes; in two there were pulmonary, in two hepatic, and in one splenic metastasis, and in one case there were numerous peritoneal growths. The mesothelial type of growths produced the most extensive metastasis and the most widespread infiltration of the body wall.

A review of the literature on renal tumors throughout the animal kingdom, which constitutes the introductory part of this article, discloses but six other cases of renal tumors in mice, all epithelial, and no other adrenal tumors.

REFERENCES

- (1) TYZZER: Jour. Med. Res., 1907, xvii, 166; 1909, xxi, 479.
- (2) HAALAND: Fourth Sci. Report, Imperial Cancer Research Fund, London, 1911, p. 1.
- (3) MURRAY: Third Sci. Report, Imperial Cancer Research Fund, London, 1908, 73.
- (4) STUMPF: Zieglers Beiträge, 1910, xlvii, 571.
- (5) MCCOY: Jour. Med. Res., 1909, xxi, 285.
- (6) WOOLLEY AND WHERRY: Jour. Med. Res., 1911, n. s. xx, 205.
- (7) BULLOCK AND ROHDENBURG: Jour. Cancer Res., 1917, ii, 39.
- (8) BRATTI: La Semana Medica, Buenos Aires, 1917, xxiv, 643.
- (9) LOEWENSTEIN: Beitr. z. klin. Chir., 1911, lxxvi, 750.
- (10) MCCOY: Jour. Infect. Dis., 1914, xiv, 53.
- (11) FOX: Jour. Path. and Bacteriol., 1912, xvii, 217.
- (12) CURTIS: Jour. Agric. Res., 1915, v, 397.
- (13) WERNICKE: Ztschr. f. Krebsforsch., 1911, x, 168.
- (14) BÜRGER: Ztschr. f. Krebsforsch., 1914, xiv, 526.
- (15) JOEST AND ERNESTI: Ztschr. f. Krebsforsch., 1915, xv, 1.
- (16) WHITE: Zool. Soc., Philadelphia, 46th Report, 1918, p. 28.
- (17) FOX: 48th Report, 1920; 49th Report, 1921.
- (18) FOX: 43rd Report, 1915; Jour. Path. and Bacteriol., 1912, xvii, 227.
- (19) FOX: Zool. Soc., Philadelphia, 42nd Report, 1914.
- (20) FOX: Jour. Path. and Bacteriol., 1912, xvii, 227.
- (21) FOX: Zool. Soc., Philadelphia, 49th Report, 1921.
- (22) SELIGMAN: Proc. London Zool. Soc., 1907, p. 173.
- (23) BAIRD: Jour. Cancer Res., 1917, ii, 103.
- (24) BORELL AND MASSON: Bull. assoc. française pour l'étude du cancer, Paris, 1911, iv, 172.
- (25) SCOTT: Jour. Cancer Res., 1917, ii, 367.
- (26) STICKER: Arch. f. klin. Chir., 1902, lxxv, 616.
- (27) McFADYEAN: Jour. Comp. Path. and Therap., 1899, xii, 139.
- (28) KIMURA: Gann, 1917, xi, 38.
- (29) TROTTER: Jour. Compt. Path. and Therap., 1911, xxiv, 1.

- (30) STEINKE: Frankfurter Ztschr. f. Path., 1910, v, 167.
- (31) ROUSSY AND WOLF: Ann. de méd., 1921, viii, 464.
- (32) CADIOT: Bull. de l'Acad. de méd., Paris, 1921, lxxxv, 396.
- (33) ROFFO: Rev. d. Inst. Bacteriol., Buenos Aires, 1918, i, 333.
- (34) MURRAY: Third Sci. Report, Imperial Cancer Research Fund, 1908, p. 41.
- (35) HAMBURGER: Virchows Arch. f. path. Anat., 1889, cxvii, 422.
- (36) WHITE: Zool. Soc., Philadelphia, 46th Report, 1918.
- (37) BLAND-SUTTON: Jour. Anat. and Physiol., 1885, xix, 464.
- (38) WELLS: Trs. Chicago Path. Soc., 1908, vii, 140.
- (39) PICK: Berl. Med. Gesellsch., 1905, ii, 406.
- (40) WILLIAMS: Natural History of Cancer, p. 11.
- (41) TEUTSCHLAENDER: Ztschr. f. Krebsforsch., 1920, xvii, 322.
- (42) KIMURA: Gann, 1920, xiv, 10.
- (43) STEINKE: Frankfurter Ztschr. f. Path., 1910, v, 167.
- (44) SMALLWOOD: Anat. Anz., 1905, xxvi, 652.
- (45) FOX: Jour. Path. and Bacteriol., 1912, xvii, 217.
- (46) FOX: Zool. Soc., Philadelphia, 49th Report, 1921.
- (47) SLYE, HOLMES, AND WELLS: Jour. Cancer Res., 1920, v, 205.
- (48) EWING: Neoplastic Diseases, 2d ed., Philadelphia 1922, p. 739.
- (49) SLYE, HOLMES, AND WELLS: Jour. Cancer Res., 1920, v, 205.
- (50) SLYE, HOLMES, AND WELLS: Jour. Cancer Res., 1919, iv, 207.
- (51) DE VECCHI AND SALMON: Quoted by EWING: Neoplastic Diseases, 2d ed., Philadelphia, 1922, p. 767.
- (52) SLYE, HOLMES, AND WELLS: Jour. Med. Res., 1914, xxx, 417.

A CRITICAL INVESTIGATION OF THE FREUND-KAMINER REACTION

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BIBLIOGRAPHY

Human tumors

In 1910, E. Freund and G. Kaminer (1) described certain phenomena occurring when tumor cells are mixed with the blood serum from non-cancerous individuals, on the one hand, or blood serum from cancer patients on the other hand. This relationship became known as the Freund-Kaminer reaction. These investigators used tumors obtained at autopsy, because they found it harder to make cell emulsions with fresh material. The healthy portions of the tumor were minced, put into a cloth and pressed out by hand in water containing 0.6 per cent NaCl plus 1 per cent NaFl; the connective tissue and blood-vessels remained behind and the cells escaped. The resulting emulsion was centrifuged and the cells were then washed two or three times with 0.6 per cent saline solution. The washed cells were finally suspended in an equal amount of 0.6 per cent saline solution, and enough NaFl was added to make the solution 1 per cent NaFl; this proportion of NaFl will preserve the cells for three weeks. In many cases the cells agglutinated during washing, so that one washing had to suffice. Organ emulsions were prepared in the same way, and freed of blood by washing once with distilled water.

The serum was obtained sometimes fresh, sometimes post-mortem; sometimes, in later experiments, one-tenth of its volume of 0.5 per cent NaFl solution was added as a preservative.

To perform the test, ten drops of serum and one drop of cell emulsion were mixed, and a drop of 0.5 per cent NaFl solution added; the whole was then well mixed, and the cells in one drop of this emulsion were counted in a Thoma-Zeiss counting chamber. The mixture was then incubated in a test tube with a rubber top at from 37°C. to 40°C., and a second count made. When there were about 20 cells to a large square, their enumeration was easy; more than this were difficult to count, and a mixture with 40 cells to the square, which gave a negative result, gave a positive result with 20 cells to the square.

Freund and Kaminer found that the serum from noncancerous subjects destroys most of the cancer cells. The experiment was controlled by incubating an emulsion of cancer cells in a corresponding amount of 0.6 per cent saline solution containing 0.3 per cent NaFl; no diminution in the number of cells was found. Non-cancerous serum did not destroy normal cells, whether these were from normal persons or from those having cancer. Heating the serum to 55°C. abolished its power to destroy cancer cells. The authors concluded that the serum of non-cancerous individuals must contain a coctolabile substance with carcinolytic powers.

In other emulsions similarly prepared, except that the serum came from cancer patients, there was no diminution in the cell content. All tests were made with the cancer cells of one patient and sera of others.

Freund and Kaminer concluded with the question: "Is the destruction of carcinoma cells by normal serum due to some property in the serum, or to some special fragility of the cancer cell itself?" and they argued that the resistance of carcinoma cells to carcinoma serum points to some lytic property in normal serum rather than to a vulnerability of the carcinoma cell.

Freund and Kaminer's paper aroused great interest, because of the diagnostic possibilities of their reaction, and the experiment was repeated by many other investigators. Thus P. v. Monakow (2) found lysis absent in 86 per cent of cases of carcinoma. A cell destruction up to 10 per cent he considered negative; between 10 per cent to 25 per cent doubtful; and above

25 per cent positive. He observed that besides cancer cells the emulsions contain connective tissue cells, which are not destroyed. In his hands only three-fourths of normal sera caused lysis of cancer cells; one quarter of the normal sera tested acted like cancer sera and one-fifth of all cancer sera destroyed cancer cells like normal sera. He, as well as Freund and Kraus, found that the reaction often failed in sarcoma.

Stammler (3) concluded that the Freund-Kaminer test showed 80 per cent positive reactions.

Ranzi (4), in 1911, in a review of diagnostic reactions for malignant tumors, concluded that the serum of cancer patients contains a substance protective for cancer cells.

Kraus and v. Graff (5) attacked the problem from a different angle. They knew from the work of Salomon and Saxl that both in pregnancy and in carcinoma the excretion of oxyprotein is increased, and that the polypeptids are increased in carcinoma and in the later months of pregnancy. On the assumption of some analogy between the two conditions, they tested the serum of pregnant women and serum from the umbilical cord, and found that cancer serum as well as that from the umbilical cord failed to dissolve cancer cells. The serum of pregnancy caused lysis of cancer cells only inconstantly, while serum drawn after confinement had no lytic action. They concluded that the placenta produces alterations in the serum causing a loss of carcinolytic qualities or the formation of a protective substance. Rabbit and guinea pig serum acts as normal human serum, and rat, goat, and sheep serum act as carcinoma serum, the former being lytic and the latter not.

Kraus, v. Graff, and Ranzi (6) also tested the reaction, and found that 71.4 per cent of tumor patients examined gave a positive reaction (lysis below 25 per cent) and 3.5 per cent a partial lysis. Of patients with other diseases and with benign tumors, 61.2 per cent gave a negative reaction, 15.3 per cent a positive reaction, and 23 per cent a partial lysis. Seven cases that had been operated on and were clinically free from recurrence gave a negative reaction (lysis over 50 per cent); hence they concluded that the Freund-Kaminer reaction is not based

on an acquired tumor disposition but is due to changes in the blood serum depending upon metabolic changes in the tumor.

Kraus and Ischiwara (7) tested embryonal cells against cancer cells, and found that human embryonal cells show even greater lysis with normal serum than do cancer cells. Furthermore, embryonal cells were cytolyzed by a cancer serum which had no action on cancer cells. There was no lysis with fetal blood serum; but maternal (retroplacental) serum was lytic.

Rosenthal (8) continued along these general lines, using a 25 per cent emulsion of cells suspended in physiological saline solution as a control. He believed that only a lysis exceeding 25 per cent can be ascribed to the action of serum. His results were as follows: Fetal cells plus pregnancy serum gave a lysis beyond 25 per cent. Fetal cells plus fetal serum showed a lysis beyond 25 per cent. Fetal cells plus umbilical cord serum gave no cytolysis.

Arzt and Kerl (9) also have attempted to determine the diagnostic value of the Freund-Kaminer reaction. They found in the group of tumors examined 83 per cent correct diagnoses and 17 per cent wrong. These diagnoses were verified by biopsy, by operation, or by autopsy. In healthy persons and in those suffering from non-tumorous diseases the reaction gave 87.5 per cent correct and 12.5 per cent incorrect diagnoses. Freund and Kaminer, when consulted about these figures, suggested that the wrong diagnoses might be due to such errors in technique as: (a) obtaining blood during the height of digestion; (b) admixture of red blood cells with the serum; (c) the use of insufficiently fresh serum; (d) the use of cells that had lost their sensitiveness.

Kraus, Ischiwara, and Winternitz (10) reached the decision that human serum can destroy cancer cells but not normal cells, and that embryonal cells behave as cancer cells in relation to adult and umbilical cord serum.

This work attracted the attention of Coca (11) who carried out more than 150 experiments, some of them in the laboratory of Freund in Vienna. All his experiments, however, even those performed in Freund's laboratory, resulted negatively,

no cytolysis being observed in any instance with fresh normal human, dog, or horse serum. He believed, therefore, that the cytolytic action of normal sera described by Freund and Kaminer depended upon some factor as yet uncontrollable, and that it cannot be made the basis of a differential test for malignant tumors.

Freund and Kaminer have recently published (12) further experiments, to show that normal serum and tissues contain an organic fatty acid able to destroy cancer cells; this they call "normal acid." Cancer and the serum of cancer patients contain an acid that protects the cancer cell by destroying the normal acid. Pending an identification of the "normal acid," an investigation was undertaken of the saturated dibasic fatty acids, the series to which they believe "normal acid" belongs, in order to see which ones are able to destroy cancer cells.

The most recent publication is that of Frankenthal (13), who found lysis of cancer cells treated with normal serum to beyond 50 per cent; but no lysis of cancer cells treated with the serum of fourteen patients having non-cancerous diseases; 25 sera of cancer patients gave 7 (28 per cent) negative results. Her conclusions are: (a) normal serum destroys cancer cells, but also normal liver cells in lesser degree; (b) cancer serum acts differently from normal serum, in that it protects the cells from destruction up to a certain degree; (c) the Freund-Kaminer reaction is neither constant nor specific and, therefore, of no diagnostic value for the present.

Animal tumors

Freund and Kaminer used tumors (as stated above) that had been in the mortuary or the laboratory for indefinite periods of time, and it is open to speculation what proportion of the cancer cells so used were alive. They also state that the serum used in their experiments was sometimes obtained fresh and sometimes postmortem, and one may justly ask whether this serum might not have been more or less decomposed. Hirschfeld and Ischiwara, working independently of each other, apparently saw this objection, for they used freshly drawn animal serum.

Animal tumors also have been employed, their great advantage being, of course that the viability of their cells can be tested by inoculation. Thus Hirschfeld (14) made a very fine emulsion of tumor cells, exposed it to the serum of non-tumor bearing animals or to that of rats with sarcoma in the proportion of 1 to 6, incubated it for three hours, shaking the mixture every twenty minutes; and then diluted it with equal parts of 0.6 per cent saline. One cubic centimeter of this suspension was inoculated into the right inguinal region of normal animals, and a series of control animals was inoculated with tumor emulsion in physiological saline solution. The tumors in the animals inoculated with tumor cells treated with normal serum were fewer and smaller than in those inoculated with tumor cells treated with tumor serum. He concluded that this experiment proves again that normal serum has a damaging action on the vitality of tumor cells, while the serum of tumor-bearing animals lacks this quality.

Finally, Ischiwara (15), working with rat sarcoma demonstrated that the cell reaction appears relatively late (thirty days) in the serum of rats, and has some relation to the size of the tumor.

The reaction has been tested from the refractometric side by Koritschoner and Morgenstern (16), and by Koritschoner alone (17).

EXPERIMENTAL

In retesting Freund and Kaminer's experimental conclusions the present writer has also retested Kraus and v. Graff's observation (5) that the serum obtained from rabbits and guinea-pigs acts on cancer cells as does normal serum; i.e., causes lysis, whereas that from rats, goats, and sheep acts like carcinoma or placenta serum, i.e., has no appreciable lytic effect. If this were proved to be correct, then the conclusions drawn from all the experiments in which normal rat serum was used (Hirschfeld and Ischiwara) would obviously have to be considered erroneous.

In a series of experiments in which blood was drawn from the heart of rats and guinea-pigs, and from an ear vein of rabbits, this observation was tested in the following manner.

The blood, drawn under aseptic precautions, was centrifuged and the resulting serum was pipetted off, care being taken not to allow any admixture of red blood cells, since Freund, in a discussion of reported failures had attributed them to faulty technique. As another suggested error was the use of serum not sufficiently fresh, all sera obtained were used immediately. Freund also attributed failure to the use of blood obtained at the height of digestion, and to avoid this the experiments now being described were made with animals which had not been recently fed.

The tumor used in making the suspension was the Flexner-Jobling rat carcinoma, since all other experiments along these lines had been carried out with rat sarcoma in spite of v. Monakow's observation, corroborated by Freund and Kaminer, that the reaction often fails with sarcoma. The tumors selected were from thirty to seventy days old, it being generally believed that transplantability and growth capacity increase, in a general way, with the age of the tumor. In no case was the skin of the animal attached to the tumor, nor were any of the growths ulcerated. Furthermore if an animal appeared sick its tumor was not used.

The tumors were extirpated under aseptic precautions and the connective tissue capsule was carefully removed, for previous observers had stated that the admixture of connective tissue vitiated the test. Thus the only connective tissue remaining was the small amount comprising the stroma.

Only the healthy margin of each tumor was used and microscopic sections were made for purposes of checking up each experiment.

A fine emulsion of the tumor was then made by thoroughly mincing it with scissors, in order that the serum might come into contact with all parts of the growth. Various machines devised for emulsifying tumors were at first used but it was found that the resultant particles were too coarse and that a much finer emulsion could be obtained by using very sharp, curved manicure scissors. All manipulations, of course, were conducted under strictly aseptic precautions.

This fine tumor emulsion was next added to the serum in the proportion of 1 to 6, and the suspension was placed in test tubes and incubated at 37°C., for three hours. Some fresh tumor emulsion was immediately transplanted, without exposure to serum, into a series of healthy laboratory rats known to be susceptible to the Flexner-Jobling carcinoma, in order to prove the viability of the tumor employed. The amount transplanted was 0.003 gram fresh tumor emulsion, which was deposited in the right inguinal region with the usual inoculating needle. The tubes placed in the incubator were each supplied with a sterile glass rod and the emulsion was vigorously stirred every half hour in order to bring all particles of the tumor emulsion thoroughly into contact with the serum and so allow the serum to exert its influence on the largest possible number of tumor cells. At the end of the three-hour period all supernatant serum was poured off and the fine particles transplanted into a series of rats.

The protocols of a few experiments selected at random from a considerable number follow:

Comparison of the number and size of the tumors in figure 1 shows no distinct difference between the control tumors and those exposed to rabbit serum. In the serum series there is found one receding tumor; but recession of the Flexner-Jobling carcinoma is a common event, and does not vitiate the results of this experiment. One inoculation was negative, but only about 80 per cent of inoculated animals, under routine conditions, grow this tumor. One animal died before the first charting. On the whole, one may say that the animals in both series had tumors of from 10 mm. to 40 mm. in diameter and that the takes are entirely comparable in both series.

Reference to figure 2 shows that in both series after forty-five days there are tumors from 10 mm. to 40 mm. in diameter, the number of growing tumors being practically 100 per cent, with the exception of one receding tumor in the serum series.

It is evident from figure 3 that the tumors were more numerous as well as larger in the serum series than in the controls.

In the next group of experiments the effect of incubation in guinea-pig serum was studied.

As may be seen from figure 4, 45 days following the inoculation there were 100 per cent takes in both series, and there is no obvious difference between the results in the two groups.

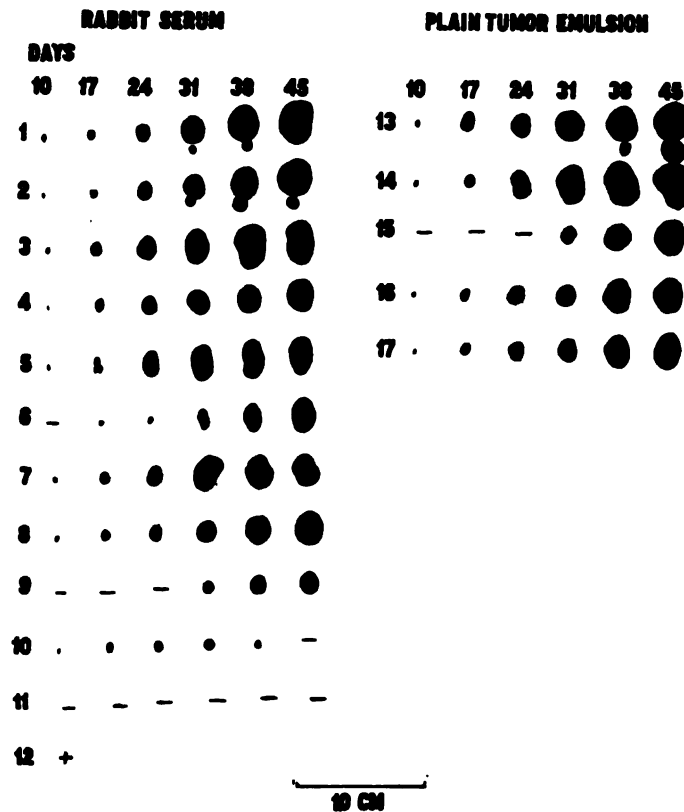


FIG. 1. EXPERIMENT $\frac{FRC}{89A}$

An emulsion of the Flexner-Jobling rat carcinoma, diluted with five parts of rabbit serum, was incubated for three hours and then transplanted into a series of 12 rats (nos. 1 to 12). Fresh tumor emulsion was inoculated into a series of 5 rats (nos. 13 to 17) to act as a control.

Reference to figure 5 will prove that there are 80 per cent takes in the serum group, whereas in the fresh tumor, or control series, there are only 40 per cent takes, and the tumors are actually smaller. Clearly the guinea-pig serum exerted no deleterious effect on the tumor cells.

It can be seen from figure 6 that forty-two days after the inoculation there were 100 per cent takes in both series and there is no obvious difference that can be observed in comparing the results in the two series.

Rabbit and guinea-pig serum may have a different action on human cells, but for the purpose of the present investigation the road is cleared by the proof that the serum from neither of these species has a deleterious effect on the tumor cells employed.

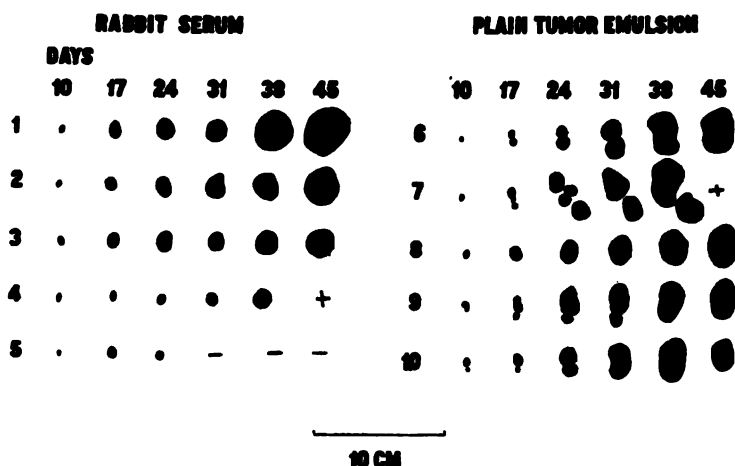


FIG. 2. EXPERIMENT $\frac{FRC}{89E}$

Five rats (nos. 1 to 5) were inoculated with an emulsion of the Flexner-Jobling rat carcinoma incubated in rabbit serum in the proportion of 1 to 6, and 5 rats (nos. 6 to 10) with fresh tumor emulsion.

The results of these experiments do not agree with Kraus and v. Graff's observation that the serum obtained from rabbits and guinea-pigs exerts a damaging action on cancer cells; for, in some instances (figs. 3 and 5) the cancer cells subjected to the action of serum from these animals grew even better than did cells from the same tumor which had not been subjected to the action of the serum. We do not attempt to explain the reason why previous observers have obtained such different results.

The objections to Freund and Kaminer's method have been already discussed. To obviate these, a series of experiments was

carried out on animal tumors because here both cells and serum can be obtained perfectly fresh, and the viability of the tumor cell can be tested by inoculation. Cells from rapidly growing rat carcinomata (Flexner-Jobling) were suspended in the serum

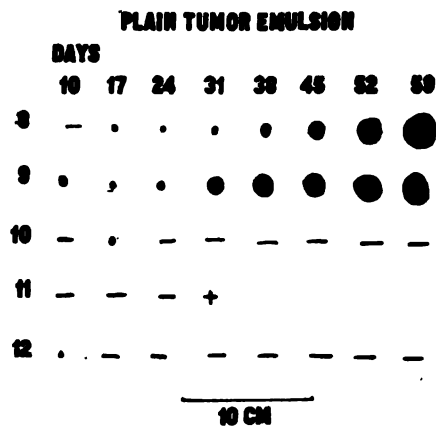
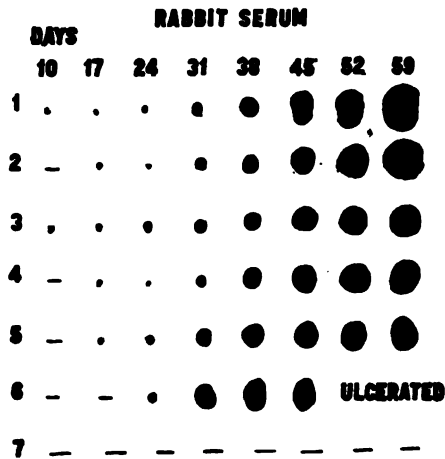


FIG. 3. EXPERIMENT $\frac{F R C}{90 K}$

A series of rats (nos. 1 to 7) was inoculated with an emulsion of the same tumor incubated in rabbit serum in the proportion of 1 to 6, and 5 rats (nos. 8 to 12) with fresh tumor emulsion to serve as a control.

of normal rats, or of rats bearing this tumor, without the intervention of any extraneous substance such as NaCl or NaFl. It was realized, of course, that only the first half of the experiment is really valid, since the serum of an animal with a trans-

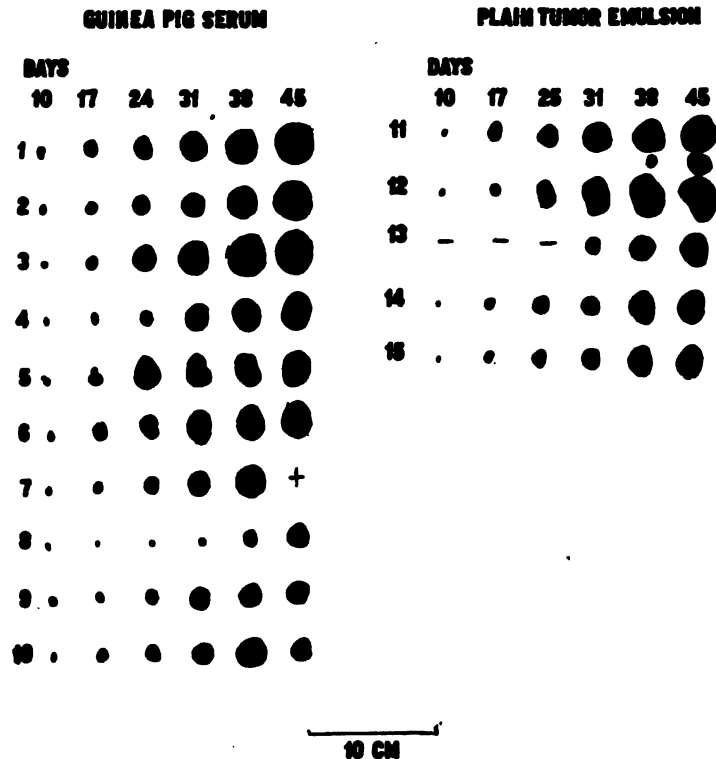


FIG. 4. EXPERIMENT $\frac{FRC}{89A}$

A series of 10 rats (nos. 1 to 10) was inoculated with an emulsion of the Flexner-Jobling rat carcinoma incubated in guinea-pig serum in the proportion of 1 to 6, and a series of 5 rats (nos. 11 to 15) with fresh tumor emulsion for controls.

planted tumor may be entirely different from that of one with a spontaneous neoplasm. But the extreme rarity of carcinoma in the rat, and the impossibility of using a sarcoma left no other course open. Mouse carcinoma was not used because of the scanty amount of serum obtainable from so small an animal.

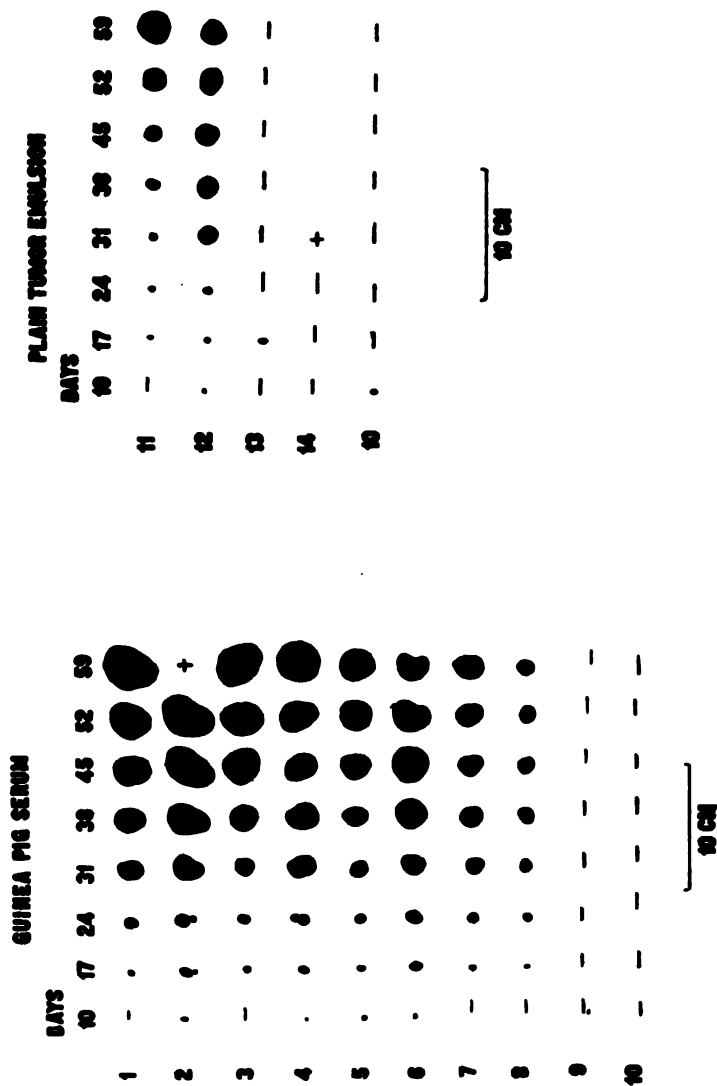


Fig. 5. EXPERIMENT 90 K

A series of 10 rats (nos. 1 to 10) was inoculated with an emulsion of the same tumor incubated in guinea-pig serum in the proportion of 1 to 6, and a series of 5 control rats (nos. 11 to 15) with fresh tumor emulsion.

Nevertheless, it can be shown whether or not normal serum damages the tumor cell; if it does not, the distinction between the serum of a normal animal and of one with a spontaneous tumor vanishes.

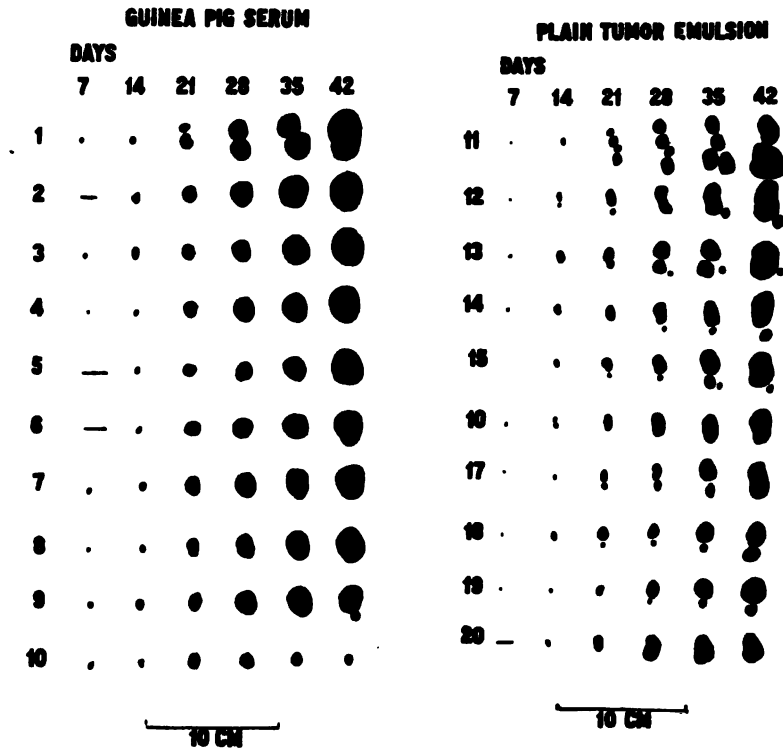


FIG. 6 EXPERIMENT $\frac{FRC}{92B}$

A series of rats (nos. 1 to 10) was inoculated with an emulsion of the Flexner-Jobling rat carcinoma incubated in guinea-pig serum in the proportion of 1, to 6, and a series of 10 rats (nos. 11 to 20) with fresh tumor emulsion for controls.

The experiments were carried out exactly as were those with rabbit and guinea-pig serum. The blood was obtained from the hearts of rats bearing Flexner-Jobling tumors from four to eight weeks old. All animals had large single or multiple tumors, ranging from 20 mm. to 35 mm. in diameter, none of which were

fixed to the skin or ulcerated. Nor did any of these animals manifest any evidence of illness.

While the other manipulations necessary to the experiment were conducted, the serum was placed in the refrigerator at 8°C. Similarly a number of rats without tumors were bled and the serum also placed in the refrigerator.

A healthy tumor was chosen, its capsule carefully stripped off, any necrotic material thoroughly scraped out, and the outer layer of the remaining healthy tumor tissue finely emulsified. All tumors used in these experiments were from thirty to ninety days old and microscopic sections of each tumor used were made and filed away for purposes of identification.

The sera were now taken from the refrigerator and to each was added this finely minced tumor in the proportion of 1 part of tumor to 5 parts of serum. A sterile glass stirring rod was added to each tube, and the tubes were incubated at 40°C. for three hours. Every half hour each tube was stirred with its glass rod in order to bring all tumor cells repeatedly into contact with the serum.

At the end of the incubation period all the serum was poured off and the fine tumor particles were transplanted into a series of rats in the manner previously described. A similar dose (0.003 gram) of fresh tumor emulsion was transplanted in the same way and at the same site without its having been exposed to any serum.

The protocols of a few typical experiments follow:

Figure 7 shows that the tumor cells incubated in normal serum grew better and more uniformly than did those incubated in tumor serum, and even better than the untreated tumor emulsion which had not been in contact with any serum whatsoever.

Figure 8 shows again that there was better growth in the series of animals inoculated with cancer cells incubated in normal serum, than in the tumor serum series. The results in the untreated tumor emulsion series were similar to those obtained in the normal serum series.

After thirty-eight days, as figure 9 shows, the results in all three series were practically alike, the tumors varying in diameter between 7 mm. and 20 mm. and growth being about the same.

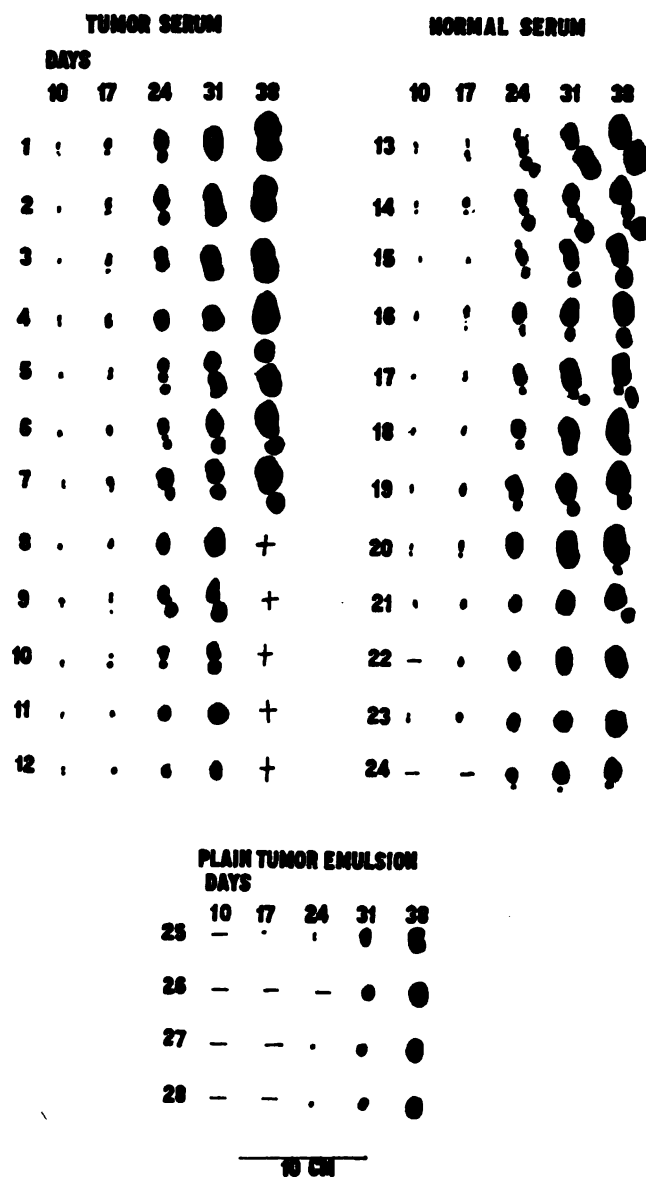


FIG. 7 EXPERIMENT $\frac{FRC}{83B}$

An emulsion diluted in the proportion of 1 to 6 with tumor serum, and in the same proportion with normal serum, was incubated and then transplanted into two series of animals, twelve each in number. Into a series of five animals a fresh undiluted tumor emulsion was transplanted.

TUMOR SERUM						NORMAL SERUM					
DAYS											
	10	17	24	31	38		10	17	24	31	38
1	13
2	14
3	15
4	16
5	17
6	18
7	-	-	.	.	.	19
8	-	-	-	-	-	20
9	-	-	-	-	-	21
10	-	-	-	-	+	22	+
11	-	-	-	+		23	+
12	+					24	.	+			

PLAIN TUMOR EMULSION					
DAYS					
	10	17	24	31	38
25	+
26	+
27	
28	+

10 6M

FIG. 8 EXPERIMENT $\frac{FRC}{84N}$

An emulsion diluted in proportion of 1 to 6 respectively with tumor serum and normal serum was incubated for three hours and then inoculated into two series of twelve animals each and the fresh tumor emulsion into a series of five animals.

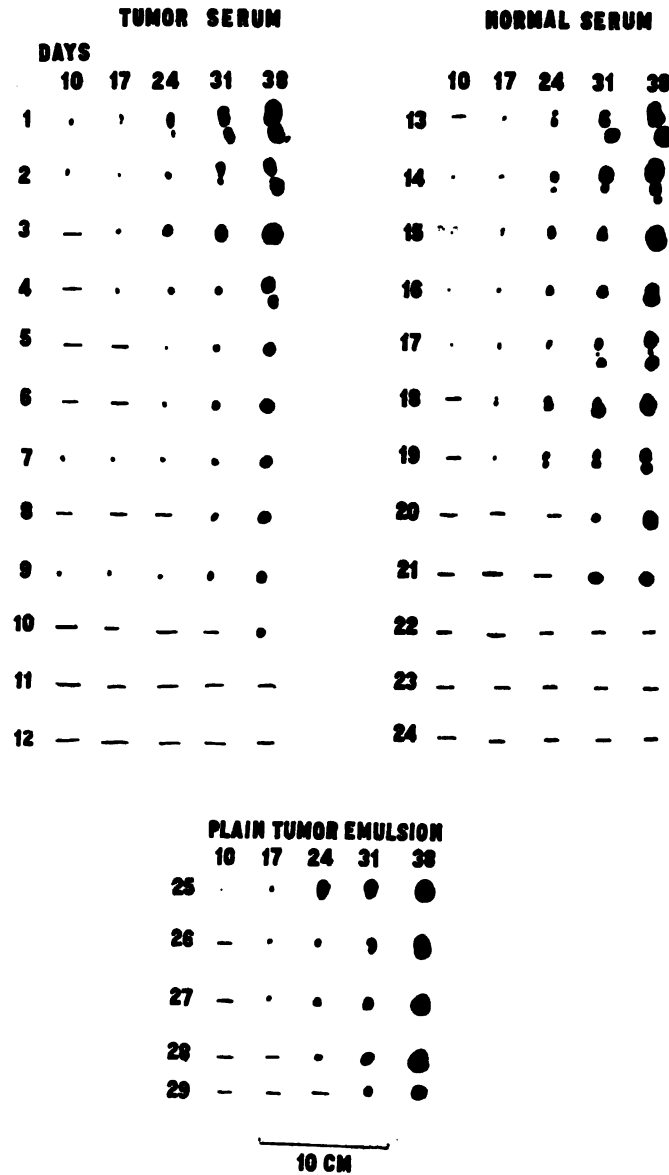


FIG. 9. EXPERIMENT $\frac{FRC}{85D}$

An emulsion diluted in the proportion of 1 to 6 respectively with tumor serum and normal serum was incubated for three hours and then inoculated into two series of twelve animals each and a fresh tumor emulsion into a series of 5 animals.

Obviously the serum of normal rats exerts no deleterious influence upon the cells of the Flexner-Jobling carcinoma, for in three separate experiments cells incubated in normal serum grew quite as well as those incubated in serum from rats with transplanted tumors. Nor was there any difference in growth between cells exposed to either serum and those transplanted without previous manipulation.

CONCLUSIONS

The conclusions to be drawn from the whole series of experiments are equally clear. After having made sure that there was no difference in the action of either rabbit or guinea-pig serum on the one hand, and rat serum on the other; and after having eliminated the sources of error enumerated by Freund and Kaminer, namely the admixture of connective tissue capsule and its blood-vessels, the presence of red blood cells in the serum, the use of old serum or of serum obtained at the height of digestion; and having, furthermore, used carcinoma (the most sensitive tumor to this test, according to the originators) instead of sarcoma, as had been done by previous investigators, we are still unable to detect any marked difference in the results of inoculation whether tumor serum or normal serum is used. The growth capacity seems neither increased in the one nor diminished in the other. We feel justified in stating, therefore, that the value of the Freund-Kaminer reaction remains at present unproved. With this proviso, however, that these experiments prove only that the serum of normal rats is devoid of any deleterious effect on the Flexner-Jobling rat carcinoma. What might be the case with serum from a rat bearing a spontaneous carcinoma, we do not yet know. However, the first half of the experiment seems sufficient; for since this shows clearly that normal serum has no harmful effect upon the cancer cell, there can be no difference, with respect to cytolytic power, between normal serum and that of an animal with a tumor, be this transplanted or spontaneous.

REFERENCES

- (1) FREUND AND KAMINER: *Biochem. Ztschr.*, 1910, xxvi, 312.
- (2) v. MONAKOW: *München. med. Wehnschr.*, 1911, lviii, 2207.

- (3) STAMMLER: Ref. München. med. Wehnschr., 1911, lviii, 1043.
- (4) RANZI: Handbuch d. Tech. & Meth. d. Imm., Ergaenzungsbd., 1911, p. 611.
- (5) KRAUS AND V. GRAFF: Wien. klin. Wehnschr., 1911, xxiv, 191.
- (6) KRAUS, V. GRAFF, AND RANZI: Wien. klin. Wehnschr., 1911, xxiv, 1003.
- (7) KRAUS AND ISCHIWARA: Wien. klin. Wehnschr., 1912, xxv, 583.
- (8) ROSENTHAL: Ztschr. f. Immunitätsforsch. u. exper. Therap., 1912, Orig., xiv, 174.
- (9) ARZT AND KERL: Wien. klin. Wehnschr., 1912, xxv, 1821.
- (10) KRAUS, ISCHIWARA, AND WINTERNITZ: Deutsch. med. Wehnschr., 1912, xxxviii, 303.
- (11) COCA: J. Cancer Res., 1917, ii, 61.
- (12) FREUND AND KAMINER: Wien. klin. Wehnschr., 1919, xxxii, 1105.
- (13) FRANKENTHAL: Ztschr. f. Krebsforsch., 1920, xvii, 250.
- (14) HIRSCHFELD: Ztschr. f. Krebsforsch., 1912, xi, 388.
- (15) ISCHIWARA: Wien. klin. Wehnschr., 1913, xxvi, 370.
- (16) KORITSCHONER AND MORGENSTERN: Biochem. Ztschr., 1920, civ, 259.
- (17) KORITSCHONER: Biochem. Ztschr., 1922, cxxix, 605.

IS CANCER A BIOLOGICAL PHENOMENON?¹

SOME HERETIC THOUGHTS ON CANCER

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The primary causes of cancer are still unknown. It seems, however, more and more evident that the development of cancer is particularly favoured by certain conditions, viz. chronic local irritants and old age.

The *chronic local irritants* are of the most variegated descriptions, belonging to all manifestations of energy—mechanic, thermic, chemical, radiological; there are also vital (animal) irritants (*Bilharzia*, *Spiroptera neoplastica* s. *Gongylonema neoplasticum*, Fibiger), and pathological irritants (precancerous conditions).

But the very difference in the nature of all these irritants indicates that it is scarcely the irritants themselves but more likely the reaction of the organism against these, which offers the conditions favourable for the development of cancer. Borrmann and Ribbert emphasize that, previous to the cancerous proliferation of the epithelium there is constantly found an inflammatory alteration of the underlying connective tissue, representing an alteration of the conditions of life of the epithelial cells.

Very frequently, though, cancer develops in localities where no irritants or irritation have been noticed.

The *influence of age* is more obvious; statistics from all countries here agree. In Norway, the mortality from malignant tumors, for the years 1901–1915 is shown in table 1.

Of these 32,274 deaths from malignant tumors, only 206, or 0.64 per cent, have occurred in persons under thirty years of age;

¹ Paper read in part before the Medical Society of Kristiania, September 21, 1921.

only 1146, or 3.55 per cent, under forty years, though these groups include 59.27 per cent and 71 per cent respectively of the whole population.

It is also remarkable that the maximum of deaths from cancer occurs in the period from sixty to seventy years of age, with nearly 30 per cent (column 6) of all cancer deaths, in spite of this period of life numbering only 5.96 per cent (column 2) of the population. And in the period from seventy to eighty years there is still an increase as to the relative importance of cancer as the cause of

TABLE 1
Cancer mortality in Norway, 1901-1915, distributed in decennial groups

(1)	(2)	(3)	(4)	(5)	(6)	(7)
AGE	POPULATION OF NORWAY 1910		CANCER MORTALITY IN NORWAY			
	Number of individuals	Per hundred of the pop- ulation	Total from 1901-1915	Average yearly	Per hun- dred of all cancer deaths	Yearly per 100,000 living
<i>years</i>						
1-20	1,063,262	45.10	38	2.53	0.12	0.024
20-30	334,091	14.17	168	11.20	0.52	0.34
30-40	275,800	11.69	940	62.66	2.91	2.27
40-50	225,181	9.56	3,226	215.06	9.99	9.55
50-60	194,109	8.24	6,925	461.66	21.45	23.73
60-70	140,579	5.96	9,658	634.85	29.93	45.80
70-80	83,825	3.56	8,462	564.15	26.22	67.30
80-90	32,551	1.38	2,716	181.00	8.41	55.60
90 and more.....	3,065	0.13	141	9.4	0.45	30.67
No age reported.....	5,327	0.19				
Total.....	2,357,790	99.99	32,274	2151	100.00	9.15

death. After the age of eighty years there seems to be some decline in the relative cancer mortality; but this is without doubt only apparent, numerous deaths from cancer in the latest decenniums of life being covered by the diagnosis of senile debility as well as by other intercurrent causes of death.

Former investigations by the Norwegian Committee for Cancer Research as well as the recent ones by Dr. Jørgen Berner make it very probable that the frequency of cancer continually increases with increasing age.

The table shows that the ages above thirty to forty years are affected by cancer proportionally 226 resp. 66 times as often as the ages below the same years.

Age must consequently be of the greatest importance for the development of cancer, and Bashford's saying, that "cancer is a function of age" contains a great amount of truth.

It must therefore be one of the nearest and most important objects for the cancer research work to investigate this influence of age.

Surely it has been hinted that age just gives the chronic irritant sufficient time to act. But this explanation of the influence of age is hardly satisfying. We do not know the time necessary for any irritant to produce cancer. But suppose that even ten or twenty years are required, there will still be every opportunity for an irritant to unfold its activity in many more thousands of cases in the twenty to forty years group, than in the older groups, but cancer is very rare at the younger age. And some of the irritants, for example, the *x*-ray, seem to be able to produce cancer after a proportionally short time and in younger individuals.

It seems more natural to attribute the influence of age upon the development of cancer to the change of metabolism in the aged. This change alters conditions of life for cells and tissues, and a local irritant may add directly and indirectly to these alterations and accentuate the influence of senile metabolism.

The influence of age on the development of cancer is so pronounced that it seems to signify a biological law. That cancer occasionally may develop in younger individuals makes no difference as to the regular influence of age. It is not the mere number of years of life that constitutes "age", but the individual's physiological condition. This senile condition may be postponed in many aged individuals; on the other side a *senilitas praecox* can be found in younger individuals, as we see it in the arteriosclerosis with young neurasthenics.

Unfortunately we do not know enough about the metabolism of age to be able to form any precise conception of its influence upon cancer formation. "Geriatrics" has not been studied as thoroughly as pediatrics.

Amongst the more important somatical peculiarities of age may be mentioned: diminution of the total mass of blood, reduction of produced and needed calories, lower temperature of the body, greater amount of cholesterin in the fat cells, increased total nitrogen in the blood serum, reduction of the number of cells in the connective tissue and of their regenerative power, atrophy of the elastic tissue, of lymph nodes, spleen, Peyer's lymphatic noduli, and of the bone marrow. Then, also, a change and diminution of the functions of the endocrine organs.

The senile changes appear at different times in different individuals as well as in the different organs of the same individual. The senile changes can occupy the entire organism (general senility) or only certain organs or parts of these (local or partial senility). Local senility can be found in the female generative organs (ovaries, uterus, and mammæ), where physiological activity and involution occur years before the manifestation of the individual's general senility.

In accordance with this fact we see that cancer of the female reproductive organs appears earlier in life than cancer in the other organs, the great majority of deaths here occurring between forty and sixty years of age (Norwegian statistics), in contrast to deaths from cancer in other organs, where the greatest numbers are to be found from sixty to eighty years of age, twenty years later. The chorionepithelioma malignum has also its greatest frequency in individuals above forty years of age (Anton Sunde).

At present we may assume that local chronic irritants, and old age, produce the altered conditions of cellular life which entail cancer.

The real nature of these conditions is still unknown to us. But it may be worth remembering, that Freund and Kaminer ten years ago have found in the blood serum and tissue fluids of healthy (non-cancerous) persons a well defined fatty acid ("normal acid"), soluble in ether and having the power of dissolving cancer cells. This acid, that has some resemblance to the decamethylen-carbonic acid $(\text{COOH})_2 (\text{C}_2\text{H}_4)_8$, of the succinic acid series, is not to be found in blood or tissue serum from cancerous individuals, whose blood has a higher alkalinity than that of non-cancerous individuals (Maud Menten).

We know from the experiments of Jacques Loeb and others that the division and multiplication of cells can be influenced by very small changes of their surrounding nutritive mediums. Similar facts are known from bacteriology.

Robertson and Burnett have been able to accelerate the growth of cancerous tumors, inoculated upon rats, by injecting the animals subcutaneously with cholesterin (and with tethelin), substances which in themselves do not produce cancer in the said animals. Luden and Bloor have also stated that the blood of cancerous patients contains more cholesterin than normally. That the fat cells in old individuals are richer in cholesterin than those in younger persons has been mentioned above.

Ferd. Blumenthal noticed that the growth of inoculated cancer in rats was accelerated when the animals got a surplus of potassium in their food, but retarded by a surplus of calcium.

Direct and local influences upon the growth of the epithelium are known since the experiments of Fischer and others with Scharlach R (and other similar substances), the application of which on the skin of rabbits produced a lively proliferation of the epithelium. And Yamagiwa and Ischikawa, and now lately Fibiger have through similar application on rats succeeded in producing growth possessing the essential qualities of the genuine carcinoma.

The local irritations and the old-age changes of metabolism entail both altered conditions of life for the cells, consequently also a change of their general manifestation of life.

But such changes in general manifestations of life are just what we find as criteria for the transformation of a normal body cell into a cancer cell: changes in outer shape and structure building power, changes in demand for nourishment, in speed of growth and proliferation, in chemical structure, enzymes and secretions (cachectic power), in sensibility towards electrical influences (x-rays), and independence of soil (metastases).

These changes of essential biological qualities in the cancer cell are just what v. Hanseemann has designated under the name of *anaplasia* (transformation), a phenomenon hitherto considered as unique, unparalleled.

But is such a transformation really so unique in the living nature?

About twenty years ago de Vries introduced into biology the phenomenon of *mutation*, the comparatively quick changes of the characteristics of species in living beings, in contrast to the very slow changes admitted by the Darwinistic principles of evolution. This mutation, first noticed by de Vries in a plant (*oenothera*), has later been described for unicellular organisms, bacteria (Weisser, Massini), algae, spirochaetae, trepanosomas, infusoria, and amebas. Thjøtta and Eide have described a paratyphoid strain that, under culture for about a month, essentially changed its character morphologically and physiologically, losing its flagelli and motility and changing into a capsulated mucus-forming bacillus; which shape it preserved through all the consecutive generations.

Gurney Dickson, in his monograph on the Transmutation of Bacteria, maintains that mutation is an adaption to altered conditions of life; it can be more or less pronounced and may include very different qualities of the bacteria: shape and size, affinity to dyes (chemical and physical properties of the protoplasm), ability of growing on a new soil, resistance against external influences, virulence, and production of ferments. Most frequently mutation occurs in cultures of old date.

There seems thus to be a *considerable parallelism between mutation and the phenomena accompanying the transformations of a normal body-cell into a cancer cell*. And the mutated cells, like the cancer cell, will transfer the newly acquired qualities to their future generations.

Through all these changes in its fundamental biological qualities the cancer cell has turned out to quite a new cellular being, foreign or even *hostile*, to the organism in which it has developed. Authors like Bashford, Abderhalden, Ribbert, Butlin and others call the cancer cell a parasite. Butlin even described it as a new and special species of beings under the name of *unicellula cancri*.

The idea that the transformation of the normal cell to a cancer cell may be a *mutation*, can certainly not explain the nature of

cancer, the nature of mutation being still enigmatical, but it brings the cancer question in a line with other biological phenomena, which may be examined and studied on a broader base; and results from other spheres of biological research may be applied upon the cancer questions.

Considering the dominating influence of age on the development of cancer, it seems necessary for the future cancer research to direct the closest attention to the metabolism of the aged. Here will be one of the future battlefields against cancer. The British *x*-ray expert, Morley Robert, thinks it essential here to produce a mental and bodily youth in the individuals. The war-cry of the medical profession in its struggle against cancer will be: Keep humanity young!

THE RELATION OF MUSCULAR ACTIVITY TO CARCINOMA¹

A PRELIMINARY REPORT

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Minneapolis, Minnesota

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Carcinoma in men, especially retired farmers, has been frequently seen by us in the past few years. These patients, men with large, well developed bodies of the "frontiersman" type, usually gave a history of having retired from the farm in good health, a few years previously, to enjoy a well deserved rest. On the other hand we have often talked with farmers, who for one reason or another still shouldered the responsibilities of the farm, who were well and working hard though sixty, seventy, or even eighty years of age. This observation brought us to consider muscular activity in relation to carcinoma.

There have been no deaths from carcinoma among the Turn Teachers (1) in the past thirty years. Thirty deaths from all causes occurred in this period, and we should expect at least three deaths from carcinoma. The Turn Teachers are of necessity active in a muscular sense. While the list is not large, the exception to the rule is interesting.

We have repeatedly noted that patients, clinically diagnosed as precancerous, improved in a most satisfactory manner on a prescription for increased daily exercise, as rope skipping for women, golf and brisk daily walks for men.

Muscular activity stimulates metabolism and retards the approach of senescence and decay. The tuberculosis problem has been greatly simplified by the recognition of the value of rest, sunshine, wholesome food and fresh air. We believe that the

¹ Read before the Hennepin County Medical Society March 6, 1922.

carcinoma problem may also be simplified by the recognition of the value of muscular activity in those periods of life most affected by carcinoma, those periods of life when we are prone to "let down" and check our pace. If muscular activity has an intimate relationship to carcinoma, the value of it will be most apparent in prophylaxis, the field which to date promises the most in the attack on this malady.

In a perusal of the literature we are unable to find any direct reference to the relation of muscular activity to carcinoma.² Hoffman (2) shows that carcinoma is world wide, is a disease of maturity, affecting both sexes and seriously affecting all civilized nations and races. Observations are quoted and statistics are presented showing that carcinoma is more frequent among the unoccupied, among people of independent means, among the leisured and professional classes, among the overnourished, well housed, well fed, and among those who live in the warmer climates. Carcinoma is less frequent among the people who are actively engaged in a gainful occupation, among the hard working classes, among those who live in large industrial centers, among peoples predominately poor and of necessity frugal, living on an alimention just sufficient for their actual needs, and among those who live in the colder climates where the struggle for existence is more difficult. Reading between the lines one common factor is noted, that of the varying degree of muscular activity. The vast majority of people follow the line of least resistance and those of independent means, the leisured and professional classes, and those who live in the tropics are not compelled to do manual labor as a means of gaining a living and hence are less liable to be active in a muscular sense. Attention must be called to Dublin's (3) conclusion with reference to the experience of the Metropolitan Life Insurance Company for 1914, 1915, 1916, that "the cancer

² It is interesting to us to find that in another part of the world similar ideas have been advanced almost simultaneously. Cherry, of Melbourne, on March 1, 1922, read a paper on A Theory of Cancer (published in Med. Jour. of Australia, April 22, abstracted in J. A. M. A., 1922, lxxix, 245) in which he attributes the recent increase of cancer to deficient muscular activity, overnutrition, and lessened skin activity, as a result of modern living conditions of civilized peoples.

mortality rate at ages where the cancer rate is significant, decreases as we go up the economic scale." To reconcile the statistics presented by Hoffman with those of Dublin one must admit that other factors than the economic status are present.

Ewing (4) states that "statistics show that sanitary measures which control infectious diseases exercise no such power over carcinoma, which is somewhat conspicuous by a relative failure to attack the poor, the overworked, the underfed, the savage, but chooses a notable proportion of its victims among the well-to-do, the well nourished, the well protected against infectious diseases, and the indolent."

Ewing has noted also that carcinoma seems to be as widespread as are animal species. The mortality from malignant tumors among pet dogs is reported to be surprisingly high. Forms of carcinoma in horses, cattle, and swine are relatively common, carcinoma of nearly every organ of fowls has been reported, mice and rats are subject to carcinoma and made use of extensively in the laboratory. It is very interesting to note that he too calls attention to the rather sharp fall in carcinoma incidence when one takes up the consideration of carcinoma in wild animals. In this respect one must also consider that wild animals have not been subjected to uniform close study, autopsies have been less frequent, and violent deaths have been more common than among the domesticated animals. McCoy (5) reports autopsy findings of 100,000 rats (95 per cent of which were *Mus norvegicus*) caught during the San Francisco rat campaign about fifteen years ago. Of these rats 103 were found to have tumors, of which 10 proved to be carcinoma. Thus he found that about one rat for every thousand had a new growth, and only 1 in 10,000 had a carcinoma, the remainder being benign tumors or sarcomata. In addition to the factors incident to the study of carcinoma among wild animals, the necessary muscular activity of the animal in its natural wild state may have a bearing on the relative infrequency of reported carcinoma.

Carcinoma has been observed under certain conditions among artificially bred fish (6). It has been clearly shown that fish kept under artificial conditions are more susceptible to the

development of carcinoma than are those living under natural conditions. We believe that the enforced inactivity of the fish in ponds and hatcheries is a potent factor in the development of carcinoma. The dietary factor must also be considered.

The present day labor saving machinery, convenient means of transportation, and ultra-conveniences of modern life all tend to make us less active physically, and it appears to us that the increasing curve of carcinoma closely approximates the inauguration of the Age of Machinery.

The recent researches into the finer biochemical changes attending the metabolism of carcinomatous patients point to the development of carcinoma as an individual biological reaction to chronic irritation, governed possibly by the previous state of metabolism. It would seem that we must look to the biochemist for information as to the exact cause of the reversion of the adult cell to fetal characteristics. The researches of Freund and Kaminer (7), Rosenthal (8), Neuberg (9), Koritschoner and Morgenstern (10), and others are very interesting in this connection.

This opportunity is taken for the presentation of a working hypothesis: That human carcinoma may be the reaction to and the result of chronic irritation of adult epithelial tissues bathed in body fluids altered by certain metabolic products as a result of deficient muscular activity.

In advancing the above hypothesis constructive and destructive criticism is invited, as by intensive cooperative effort sufficient data pro and con will be obtained definitely to prove or disprove our contention.

An investigation in an experimental way of some of the phases of muscular activity and its relation to carcinoma is being conducted which will be reported in the near future. We refer especially to the investigation of the effect of muscular activity on experimental carcinoma in mice. We are also preparing to ascertain the effect on human carcinoma of horse serum obtained after graduated amounts of muscular activity.

In an effort at this time to gain some concrete evidence bearing on the subject, a study of carcinoma in Minnesota for the past

three years was undertaken. Access to original certificates of death was obtained through the courtesy of the Bureau of Vital Statistics, Minnesota State Board of Health, there being no compilations available. Among 86,838 records examined 6,351 deaths were ascribed to malignant disease for the years 1918, 1919, and 1920. Sarcoma was named as cause of death in 452 records. There were 3135 deaths from carcinoma among males, and these were used as the basis for the present study. Carcinoma among females was not studied at this time because of the difficulty in estimating the amount of muscular activity relative to occupations of this sex. The deaths were tabulated according to occupation and the occupations divided by us into six groups according to our idea of the muscular activity necessary to that occupation. It is to be regretted that no scientific criterion was available for this classification. Some method as suggested by Waller and DeDecker (11) might be applicable if standardized by sufficient usage. We have tried to use our best judgment in each case. A special report was obtained from the Bureau of Census on the number of persons engaged in each occupation based on the Census of 1920. The deaths in each group for three years were totalled, averaged, the number of persons engaged calculated and the death rate computed on the basis of 100,000 persons engaged to bring each group to a common ground for comparison. The death rates are in a measure not absolutely reliable because of the short period of time under consideration and the present day laxness of the average undertaker in completely filling out certificates of death. We grouped the occupations as follows: Group 1. Occupations involving great muscular activity. In this group are included stonecutters, blacksmiths, boilermakers, moulders and the like. Group 2. Occupations involving moderate amounts of muscular activity, as carpenters, cabinet makers, common laborers, masons, plumbers, and the like. Group 3. Includes occupations involving medium amounts of muscular activity as foremen, millers, brewers, agents, printers, etc. Group 4. Occupations involving small amounts of muscular activity, as the professions, office workers, and the like. Group 5. The farmers, because of the

seasonal character of their work in Minnesota, were classed alone. Group 6. This group includes those not actively engaged in any gainful occupation, students, idiots, epileptics, inmates of insane hospitals, and those who have retired from gainful occupations.

Hoffman (12) reports the following statistics:

Death rate per 100,000 males, England-Wales, thirty-five years of age and over

	1890-1892	1900-1902
All males.....	165.5	210.3
Occupied.....	146.0	180.0
Not occupied.....	359.0	503.8

The differences in mortality are very striking and we believe they may be explained on the basis of muscular activity.

In Minnesota for the years 1918, 1919, 1920 we have found the death rate per 100,000 males as follows:

1. Those 21 years of age and over:
 - a. Active (groups 1, 2, 3, 4, 5)..... 103.5
 - b. Inactive (group 6)..... 510.0
2. Those 45 years of age and over:
 - a. Active (groups 1, 2, 3, 4, 5)..... 235.8
 - b. Inactive (group 6)..... 681.0

The figures for Minnesota are seen to parallel roughly those for England and Wales (see chart 1).

In a consideration of the age class 21 years of age and over we have found the death rate from carcinoma lowest in group 1 (in which the necessary muscular activity is greatest) and highest in group 6 (in which the necessary muscular activity is least). There is a stairstep gradation between the extremes. The ratios between the death rates in the various groups may be thus expressed: I:II:III:IV:V:VI: 1::1:1.7: 2.1:2.3:3.6: 11.8 (see chart 2, table 1).

To ascertain the ratios in the various age classes we have subdivided the active groups:

A. Those twenty-one to forty-four years of age. In this age class the ratios may be expressed thus: I:II:III:IV:V::1:1.4: 1.5:2.2:2.0 (see table 2, chart 3).

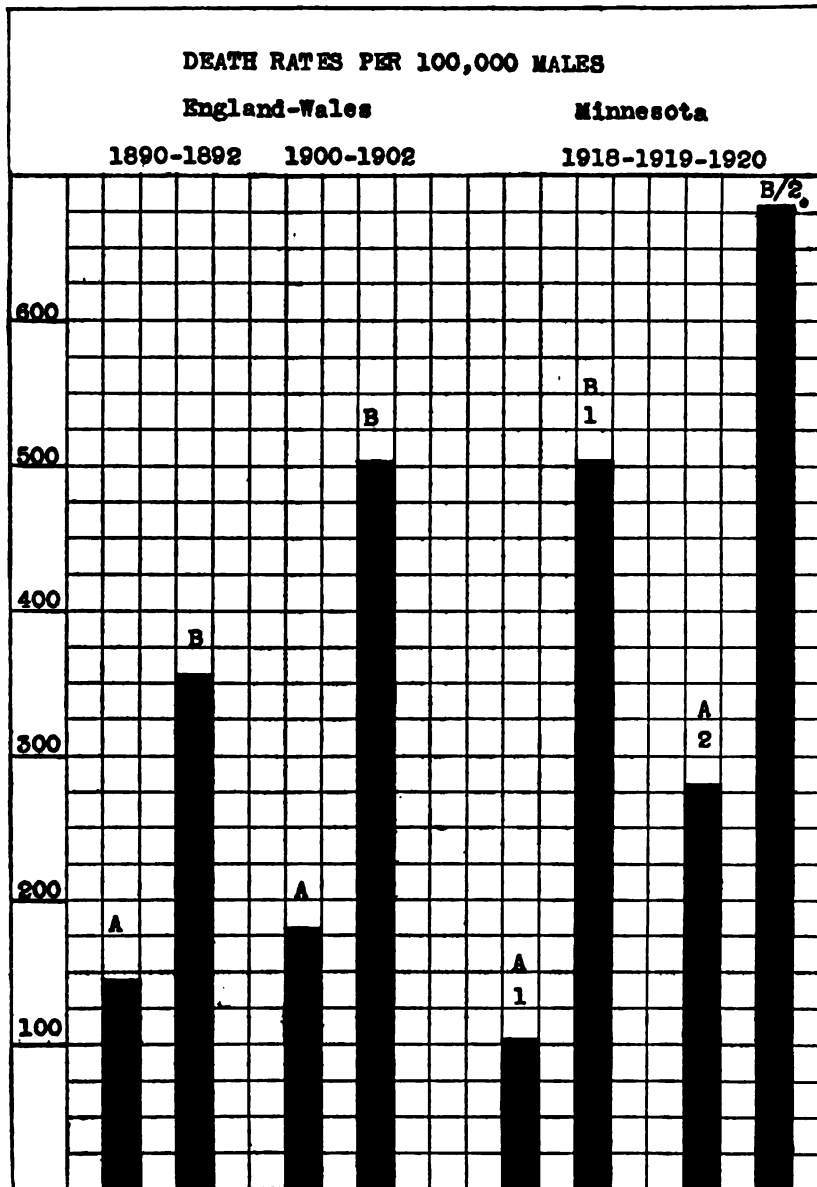


CHART 1. Columns lettered *A* denote occupied; *B*, not occupied; columns not numbered denote those thirty-five years of age and over; numbered *1*, twenty-one years of age and over; *2*, forty-five years of age and over.

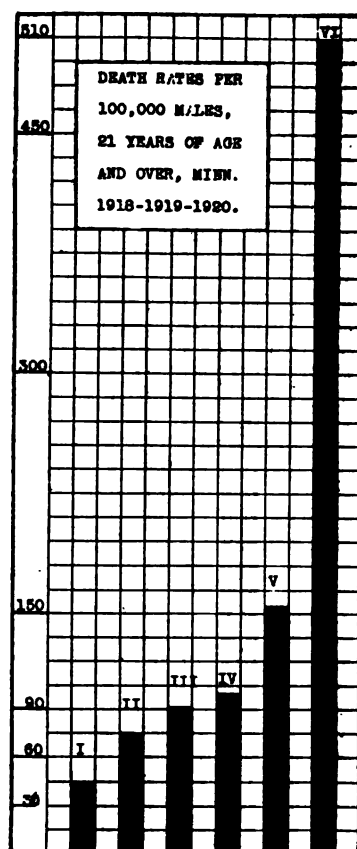


CHART 2. COLUMNS NUMBERED I, II, ETC., REFER TO OCCUPATION GROUPS 1, 2, ETC.

TABLE 1
Death rates per 100,000 males, twenty-one years of age and over, Minnesota, 1918, 1919, 1920

GROUP	NUMBER OF PERSONS	DEATHS	DEATH RATE
1	46,736	20.0	43.0
2	168,460	128.0	75.8
3	84,523	77.0	91.1
4	211,824	213.9	100.8
5	170,483	267.7	156.6
1,2,3,4,5	682,026	706.4	103.5
6	64,503	330.0	510.0

B. Those forty-five to sixty-four years of age. The ratios existing are I:II:III:IV:V::1:1.8:2.1:2.0:2.0 (see chart 4, table 3).

C. In those sixty-five years of age and over we have found the difference less striking as might be expected for this age class, the ratios being: I:II:III:IV:V::1:1.5:1.1:1.4:1.9 (see table 4, chart 5).

D. Considering those of another age class, from twenty-one to sixty-four years of age, we find there is progressive increase in death rate with progressive diminution in amount of necessary muscular activity. The ratios may be expressed: I:II:III:IV:V::1:1.6:2.3:2.4:3.1 (see table 5, chart 6).

In all the age classes considered the death rate from carcinoma among those actively engaged in a gainful occupation is roughly inversely proportional to the amount of muscular activity necessary to that occupation. The only out and out exception to the rule is in group 5, the farmers, for which we lack a satisfactory explanation except that the seasonal character of the occupation in Minnesota may have a bearing, in that during the spring and fall seasons the work is very heavy, while in the winter and summer, especially in winter, the work is very light.

The experience of the Industrial Department of the Metropolitan Life Insurance Company (13) discloses that cancer causes the following percentage of total deaths for the following occupations:

Group 1. Blacksmiths 7.6 per cent; miners 4.6; moulders 3.9; average for the group 5.9 per cent.

Group 2. Laborers 5.5; longshoremen 4.3; machinists 4.5; masons 6.5; average 5.2 per cent.

Group 3. Teamsters 3.6; printers 2.7; painters 4.1; track laborers 4.7; average 3.8 per cent.

Group 4. Clerks 3.1; cigarmakers 5.2; average 4.2 per cent.

Group 5. Farmers 7.6 per cent.

The above figures do not indicate any direct influence of the occupational disease factor.

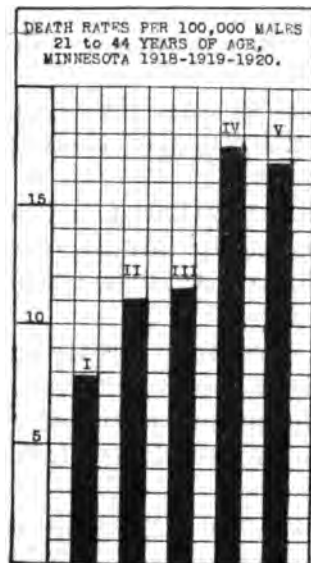


CHART 3

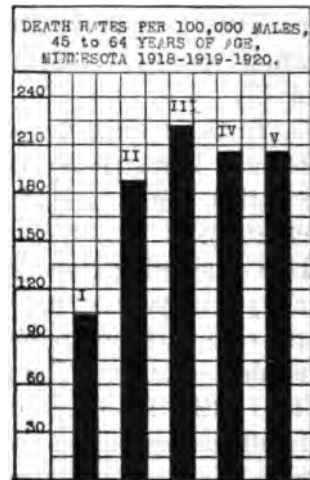


CHART 4

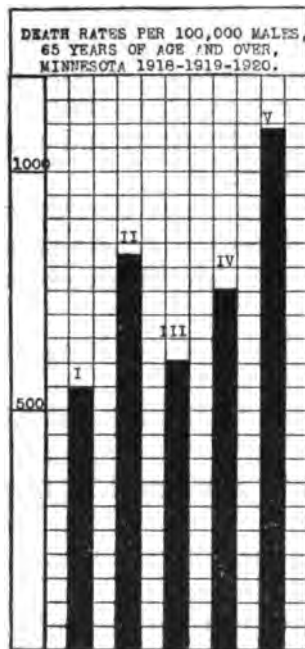


CHART 5

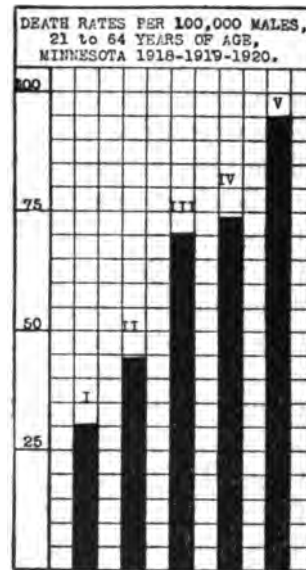


CHART 6

CHART 3. COLUMNS NUMBERED I, II, ETC., REFER TO GROUPS 1, 2, ETC.

CHART 4. COLUMNS NUMBERED I, II, ETC., REFER TO GROUPS 1, 2, ETC.

CHART 5. COLUMNS NUMBERED I, II, ETC., REFER TO GROUPS 1, 2, ETC.

CHART 6. COLUMNS NUMBERED I, II, ETC., REFER TO GROUPS 1, 2, ETC.

TABLE 2

Death rates per 100,000 males engaged, twenty-one to forty-four years of age, Minnesota 1918, 1919, 1920

GROUP	NUMBER OF PERSONS	DEATHS	DEATH RATE
1	34,587	2.7	7.8
2	128,233	14.3	11.1
3	58,362	6.7	11.5
4	141,902	24.7	17.4
5	93,661	15.7	16.7

TABLE 3

Death rates per 100,000 males engaged, forty-five to sixty-four years of age, Minnesota, 1918, 1919, 1920

GROUPS	NUMBER OF PERSONS	DEATHS	DEATH RATE
1	11,057	11.3	102.2
2	4,373	65.3	189.9
3	22,955	51.0	221.1
4	61,321	126.0	205.5
5	66,168	136.0	205.5

TABLE 4

Death rates per 100,000 males engaged, sixty-five years of age and over, Minnesota, 1918, 1919, 1920

GROUPS	NUMBER OF PERSONS	DEATHS	DEATH RATE
1	1,092	6.0	549.4
2	5,854	48.3	825.0
3	3,206	19.3	602.0
4	8,601	65.0	755.7
5	10,654	115.7	1065.0

TABLE 5

Death rates per 100,000 males engaged, twenty-one to sixty-four years of age, Minnesota, 1918, 1919, 1920

GROUPS	NUMBER OF PERSONS	DEATHS	DEATH RATE
1	45,644	14.0	30.6
2	162,606	79.7	49.0
3	81,317	57.7	70.9
4	203,223	148.7	73.1
5	150,829	151.7	94.9

A tabulation of the ages at death in the different occupation groups discloses a rather close approximation of extremes and averages so it appears the differences in death rates shown above can not be wholly explained on a basis of age incidence; that is, there is not sufficient difference between the ages of those in the different occupation groups to explain the ratios found to exist between the death rates in the various groups of occupations (see table 6).

TABLE 6
Ages at death, males, Minnesota, 1918, 1919, 1920

GROUPS	NUMBER OF DEATHS	EXTREMES	AVERAGE AGE AT DEATH
1	67	24-81	57.3
2	379	29-91	60.3
3	201	29-83	57.7
4	667	24-88	56.8
5	878	25-90	62.2
6	984	27-103	70.4

SUMMARY

In a preliminary report attention is called to a new factor in carcinoma etiology. Carcinoma has been frequently observed in retired farmers, seldom in active individuals. Precancerous patients have improved clinically with increased muscular activity. Attention is called to observations of less carcinoma among those of necessity physically active. The reported incidence among domesticated animals is greater than in wild animals. A working hypothesis is advanced: That human carcinoma may be the reaction to and the result of chronic irritation of adult epithelial tissue bathed in body fluids altered by certain metabolic products as a result of deficient muscular activity. From a study of carcinoma deaths among males in Minnesota for three years it appears that the death rate in those who are active is greatly exceeded by the death rate in those who are inactive. From a study of the death rates of those who are actively engaged in a gainful occupation it appears that the death rate is lowest in those occupations involving the greatest amounts of necessary muscular activity, and is highest in those occupations involving the least amounts of muscular activity. The age

incidence factor of the cases studied does not explain the variations shown. The figures from the experience of the Metropolitan Life Insurance Company do not show any marked influence of occupational disease on carcinoma death rate. Additional study and accumulation of data are necessary to establish definitely the status of the relation of muscular activity to carcinoma.

CONCLUSIONS

1. Carcinoma constitutes a serious menace to the adults of all civilized races.
2. It appears that the recent increase has accompanied the advent of the Age of Machinery.
3. The reported incidence among the lower animals appears inversely proportional to the degree of muscular activity necessary to the existence of the animal.
4. The death rate in males actively engaged in a gainful occupation is less than the death rate in those not actively engaged in any gainful occupation.
5. The death rate among males actively engaged in a gainful occupation is inversely proportional to the degree of muscular activity necessary for that occupation.
6. A new factor in carcinoma etiology is proposed.
7. A working hypothesis is suggested.

REFERENCES

- (1) Personal Communication from the Secretary of the Turn Teachers Mutual Benefit Association.
- (2) HOFFMAN, F. L.: *The Mortality from Cancer Throughout the World*. Newark, 1915.
- (3) DUBLIN, L. I.: *Mortality Statistics of Insured Wage Earners and their Families*. Metropolitan Life Insurance Company, 1919, Chapter ix.
- (4) EWING, J.: *Animal Experimentation and Cancer*. *Defense of Research*, Pamphlet iv, A. M. A., 1911, pp. 4-6.
- (5) MCCOY, G. W.: A preliminary report on tumors found in wild rats. *Jour. Med. Res.*, 1909, xvi, 285.
- (6) BAINBRIDGE, W. S.: *The Cancer Problem*. New York, 1914, p. 55.
- (7) FREUND, E., AND KAMINER, G.: Reactions between tumor cells and blood serum. *Biochem. Ztschr.*, 1910, xxvi, 312.
- (8) ROSENTHAL, E.: The biological parallel between cancer and fetal cells. *Ztschr. f. Immunitätsforsch. u. exper. Therap.*, 1912, xiv, 174.

- (9) NEUBERG, C.: Chemistry of tumors. *Biochem. Ztschr.*, 1910, xxvi, 344.
- (10) KORITSCHONER, I. R., AND MORGENSTERN, O.: Refractometric studies of the Freund-Kaminer reaction. *Biochem. Ztschr.*, 1920, civ, 259.
- (11) WALLER, A. D., AND DeDECKER, G.: The physiological cost of muscular work. *Brit. Med. Jour.*, 1921, i, 669; 1921, ii, 627.
- (12) HOFFMAN, F. L.: The mortality from cancer in different occupations. *Ztschr. d. osterr. Sanitätswesen*, 1918, supplement to 1-26, pp. 33-42.
- (13) DUBLIN, L. I.: Causes of death by occupation. *Bull. U. S. Bureau Labor Statistics*, No. 207, U. S. Government Printing Office, 1917.

INDEX

Activity, muscular, The relation of, to carcinoma. A preliminary report....	365
Adenoma, atypical, An, of the pancreas originating in islet tissue.....	277
Adrenal, Primary spontaneous tumors in the kidney and of mice.....	305
Age, Teratomas and their relation to.....	261
Albino rats, The influence of certain diets upon tumor susceptibility and growth in (summary).....	100
Animal tumors, transplanted, The effect of combined heat and radiation upon (summary).....	101
Animals, immune, Effect of blood from, upon transplantable tumors.....	25
Bagg, Halsey J., Interstitial injections of an active deposit of radium emanation in a rat carcinoma (summary).....	104
Benedict, Stanley R., and Sugiura, K., The influence of certain diets upon tumor susceptibility and growth in albino rats (summary).....	100
Bernhard, A., Rohdenburg, G. L., and Krehbiel, O. F., Further investigations of disturbances of blood sugar equilibrium in their relation to neoplasia..	223
Biological phenomenon, Is cancer a. Some heretic thoughts on cancer.....	357
Biology, tumor, A phase of (summary).....	96
Bladder, cancer of the, Radium in (summary).....	190
Blood, Effect of, from immune animals upon transplantable tumors.....	25
— sugar equilibrium, Further investigations of disturbances of, in their relation to neoplasia.....	223
—, The protein content of the whole, and plasma in cancer.....	127
Bovie, William T., Further experiments on sensitisation to heat by radiation (summary).....	193
Breast, cancer of the, A few late results after the radical operation for (summary).....	88
Bulkley, L. Duncan, Proofs of the constitutional nature of cancer (summary)	185
Burrows, Montrose T., Problems in cancer research.....	191
Burrows, Montrose T., Problems in cancer research.....	131
Cancer and parasite.....	257
—, Is, a biological phenomenon? Some heretic thoughts on cancer	357
—, mortality, Is, increasing?.....	251
—, of the bladder, Radium in (summary).....	190
—, of the breast, A few late results after the radical operation for (summary)	88
—, operations, End-result reports of (summary).....	187
—, Proofs of the constitutional nature of (summary).....	185
—, research, Problems in.....	191
—, research, Problems in.....	131
—, Research, Proceedings of the American Association for, Fourteenth Annual Meeting.....	175

Cancer, Research, Proceedings of the American Association for, Thirteenth Annual Meeting.....	87
—, The protein content of the whole blood and plasma in.....	127
Carcinoma, A comparison of the growth of mice which ultimately develop, with the growth of mice which do not develop carcinoma.....	7
—, Primary spontaneous squamous cell, in mice. Studies on the incidence and inheritability of spontaneous tumors in mice.....	57
—, rat, Interstitial injections of an active deposit of radium emanation in a (summary).....	104
—, squamous-cell, The occurrence of, in mice (summary).....	180
—, The influence upon the growth of transplanted Flexner-Jobling rat, of hydrogen ions and of various salts in different concentrations.....	285
—, The relation of muscular activity to. A preliminary report.....	365
Cell, fat, A study of a lipomyxosarcoma with comments upon the origin of the.....	109
Chromatophores, Inoculation of sarcomatous tumors into negro fowls, with special reference to the significance of.....	31
Dahlstrom, A. W., and Sivertsen, Ivar, The relation of muscular activity to carcinoma. A preliminary report.....	365
Diets, The influence of certain, upon tumor susceptibility and growth in albino rats (summary).....	100
Duane, William, High frequency x-ray spectra.....	179
Falk, K. George, Sugiura, Kanematsu, and Noyes, Helen Miller, The influence upon the growth of transplanted Flexner-Jobling rat carcinoma of hydrogen ions and of various salts in different concentrations.....	285
Fat cell, A study of a lipomyxosarcoma with comments upon the origin of the.....	109
Flexner-Jobling rat carcinoma, The influence upon the growth of transplanted, of hydrogen ions and of various salts in different concentrations.....	285
Fowls, negro, Inoculation of sarcomatous tumors into, with special reference to the significance of chromatophores.....	31
Freund-Kaminer reaction, A critical investigation of the.....	337
Fujii, Tamotsu, and Kon, Yutaka, Inoculation of sarcomatous tumors into negro fowls, with special reference to the significance of chromatophores.....	31
Gade, F. G., Is cancer a biological phenomenon? Some heretic thoughts on cancer.....	357
Goldblatt, Harry, An atypical adenoma of the pancreas originating in islet tissue.....	277
Greenough, Robert B., and Simmons, Channing C., End-result reports of cancer operations (summary).....	187
Growth of mice, A comparison of the, which ultimately develop carcinoma with the growth of mice which do not develop carcinoma.....	7
—, of transplanted Flexner-Jobling rat carcinoma, The influence upon the, of hydrogen ions and of various salts in different concentrations.....	285
—, rate of transplanted spontaneous tumors in mice, Effect of a reduction of lymphocytes on.....	1

Heat and radiation, combined, The effect of, upon transplanted animal tumors (summary).....	101
—, sensitization to, Further experiments on, by radiation (summary).....	193
Heredity, The influence of, in determining tumor metastasis.....	139
—, The influence of, in determining tumor metastasis (summary).....	182
Herly, Louis, A critical investigation of the Freund-Kaminer reaction.....	337
Heterosexual characters, Relation of, to neoplasms and developmental errors (summary).....	186
Himwich, H. E., Teratomas and their relation to age.....	261
Holmes, Harriet F., Slye, Maud, and Wells, H. Gideon, Primary spontaneous squamous cell carcinomas in mice. Studies on the incidence and inheritability of spontaneous tumors in mice. Fifteenth communication..	57
—, Slye, Maud, and Wells, H. Gideon, Primary spontaneous tumors in the kidney and adrenal of mice. Studies on the incidence and inheritability of spontaneous tumors in mice. Seventeenth communication.....	305
—, Slye, Maud, and Wells, H. Gideon. Primary spontaneous tumors of the ovary in mice. Studies in the incidence and inheritability of spontaneous tumors in mice. Fourteenth report (summary).....	92
—, Slye, Maud, and Wells, H. Gideon, The occurrence of squamous-cell carcinoma in mice (summary).....	180
Huffman, Otto V., Relation of heterosexual characters to neoplasms and developmental errors (summary).....	186
Hydrogen ions, The influence upon the growth of transplanted Flexner-Jobling rat carcinoma of, and of various salts in different concentrations.	285
Inoculation of sarcomatous tumors into negro fowls, with special reference to the significance of chromatophores.....	31
Ions, hydrogen, The influence upon the growth of transplanted Flexner-Jobling rat carcinoma of, and of various salts in different concentrations....	285
Islet tissue, An atypical adenoma of the pancreas originating in.....	277
Jacobson, Victor C., A study of a lipomyxosarcoma with comments upon the origin of the fat cell.....	109
Kellert, Ellis, Influence of the lymphocyte on the peritoneal implantation of sarcoma in mice.....	41
Kidney and adrenal of mice, Primary spontaneous tumors in the.....	305
Knox, L. C., Massage and metastasis (summary).....	192
Kon, Yutaka, and Fujii, Tamotsu, Inoculation of sarcomatous tumors into negro fowls, with special reference to the significance of chromatophores.	31
Krehbiel, O. F., Rohdenburg, G. L., and Bernhard, A., Further investigations of disturbances of blood sugar equilibrium in their relation to neoplasia..	223
Kross, Isidor, Cancer and parasite.....	257
—, Effect of blood from immune animals upon transplantable tumors....	25
—, Parabiosis and tumor growth.....	121
—, Pregnancy and tumor growth.....	245
Levin, Isaac and Levine, Michael, The rôle of neoplasia in parasitic diseases of plants.....	95

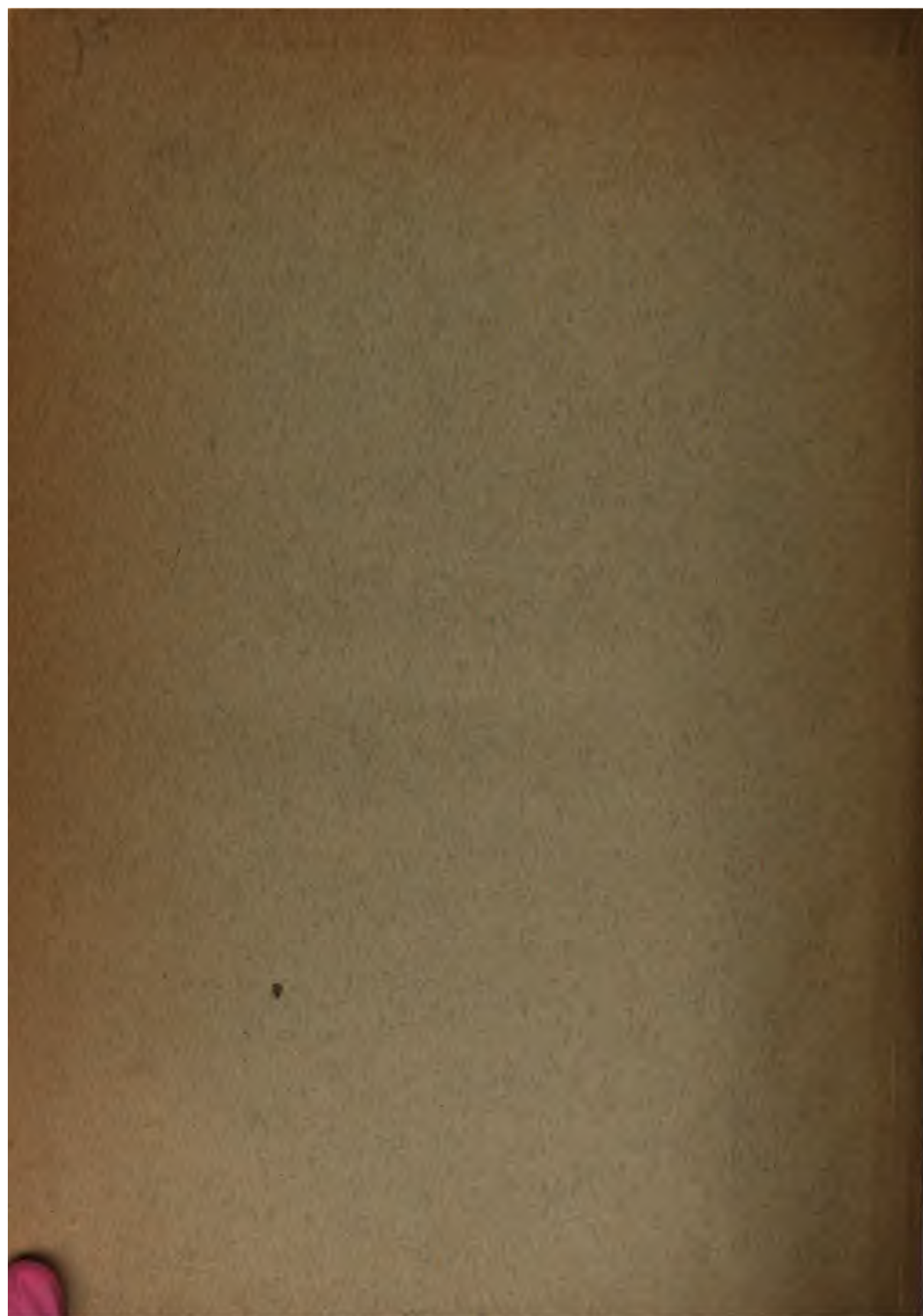
Levine, Michael, and Levin, Isaac, The rôle of neoplasia in parasitic diseases of plants.....	95
Lipomyxosarcoma, A study of a, with comments upon the origin of the fat cell.....	109
Little, C. C., Factors underlying susceptibility to a transplantable tumor in mice (summary).....	106
Loeb, Leo, Further investigations on the origin of tumors in mice. VII. Tumor age and tumor incidence.....	197
Lymphocyte, Influence of the, on the peritoneal implantation of sarcoma in mice.....	41
Lymphocytes, Effect of a reduction of, on the growth rate of transplanted spontaneous tumors in mice.....	1
Massage and metastasis (summary).....	192
Metastases, tumor, The influence of heredity in determining.....	139
Metastasis, Massage and (summary).....	192
—, tumor, The influence of heredity in determining (summary).....	182
Meyer, Willy, A few late results after the radical operation for cancer of the breast (summary).....	88
Mice, A comparison of the growth of, which ultimately develop carcinoma with the growth of mice which do not develop carcinoma.....	7
—, Effect of a reduction of lymphocytes on the growth rate of transplanted spontaneous tumors in.....	1
—, Factors underlying susceptibility to a transplantable tumor in (summary).....	106
—, Further investigations on the origin of tumors in.....	197
—, Influence of the lymphocyte on the peritoneal implantation of sarcoma in.....	41
—, Primary spontaneous squamous cell carcinomas in. Studies on the incidence and inheritability of spontaneous tumors in mice.....	57
—, Primary spontaneous tumors in the kidney and adrenal of.....	305
—, Primary spontaneous tumors of the ovary in (summary).....	92
—, Studies in the incidence and inheritability of spontaneous tumors in....	139
—, Studies in the incidence and inheritability of spontaneous tumors in (summary).....	182
—, The occurrence of squamous-cell carcinoma in (summary).....	180
Muscular activity, The relation of, to carcinoma. A preliminary report ...	365
Negro fowls, Inoculation of sarcomatous tumors into, with special reference to the significance of chromatophores.....	31
Neoplasia, Further investigations of disturbances of blood sugar equilibrium in their relation to.....	223
—, The rôle of, in parasitic diseases of plants.....	95
Neoplasms, Relations of heterosexual characters to, and developmental errors (summary).....	186
Noyes, Helen Miller, Sugiura, Kanematsu, and Falk, K. George, The influence upon the growth of transplanted Flexner-Jobling rat carcinoma of hydrogen ions and of various salts in different concentrations.....	285

Operations, cancer, End-result reports of (summary).....	187
Ovary, Primary spontaneous tumors of the, in mice (summary).....	92
Pancreas, An atypical adenoma of the, originating in islet tissue.....	277
Parabiosis and tumor growth.....	121
Parasite, Cancer and.....	257
Parasitic diseases of plants, The rôle of neoplasia in.....	95
Peritoneal implantation of sarcoma in mice, Influence of the lymphocyte on the.....	41
Phenomenon, biological, Is cancer a, Some heretic thoughts on cancer.....	357
Plants, The rôle of neoplasia in parasitic diseases of.....	95
Plasma, whole blood and, The protein content of the, in cancer.....	127
Pregnancy and tumor growth.....	245
Prime, Frederick, A phase of tumor biology (summary).....	96
—, Effect of a reduction of lymphocytes on the growth rate of transplanted spontaneous, tumors in mice.....	1
—, and Rohdenburg, George L. The effect of combined heat and radiation upon transplanted animal tumor (summary).....	101
—, and Wood, F. C., Further studies in radiation dosage (summary).....	177
Proceedings of the American Association for Cancer Research, Thirteenth Annual Meeting.....	87
Report of the council.....	87
A few late results after the radical operation for cancer of the breast....	88
Primary spontaneous tumors of the ovary in mice. Studies in the incidence and inheritability of spontaneous tumors in mice. Fourteenth report...	92
The rôle of neoplasia in parasitic diseases of plants.....	95
A phase of tumor biology.....	96
The influence of certain diets upon tumor susceptibility and growth in albino rats.....	100
The effect of combined heat and radiation upon transplanted animal tumors.....	101
Interstitial injections of an active deposit of radium emanation in a rat carcinoma.....	104
Factors underlying susceptibility to a transplantable tumor in mice.....	106
Proceedings of the American Association for Cancer Research, Fourteenth Annual Meeting.....	175
Report of the council.....	175
Report on the JOURNAL OF CANCER RESEARCH.....	177
Further studies in radiation dosage.....	177
High frequency x-ray spectra.....	179
The occurrence of squamous-cell carcinoma in mice.....	180
The influence of heredity in determining tumor metastasis. Studies in the incidence and inheritability of spontaneous tumors in mice. Fif- teenth report.....	182
Proofs of the constitutional nature of cancer.....	185
Relation of heterosexual characters to neoplasms and developmental errors.....	186
End-result reports of cancer operations.....	187
Radium in cancer of the bladder.....	190

Proceedings of Fourteenth Annual Meeting— <i>continued</i>	
Problems in cancer research.....	191
Massage and metastasis.....	192
Further experiments on sensitization to heat by radiation.....	193
Malignant tumors of the thyroid.....	194
Experimental production of tumors.....	196
Protein content, The, of the whole blood and plasma in cancer.....	127
Radiation, combined heat and, The effect of, upon transplanted animal tumors (summary).....	
—, dosage, Further studies in (summary).....	177
—, Further experiments on sensitization to heat by (summary).....	193
Radium emanation, Interstitial injections of an active deposit of, in a rat carcinoma (summary).....	104
—, in cancer of the bladder (summary).....	190
Rat carcinoma, Flexner-Jobling, The influence upon the growth of transplanted, of hydrogen ions and of various salts in different concentrations.	285
—, carcinoma, Interstitial injections of an active deposit of radium emanation in a (summary).....	104
Rats, albino, The influence of certain diets upon tumor susceptibility and growth in (summary).....	100
Ray, L. A., and Robertson, T. Brailsford., A comparison of the growth of mice which ultimately develop carcinoma with the growth of mice which do not develop carcinoma.....	8
Reaction, Freund-Kaminer, A critical investigation of the.....	337
Robertson, T. Brailsford, and Ray, L. A., A comparison of the growth of mice which ultimately develop carcinoma with the growth of mice which do not develop carcinoma.....	7
Rohdenburg, George L., and Prime, Frederick. The effect of combined heat and radiation upon transplanted animal tumors (summary).....	101
Rohdenburg, G. L., Krehbiel, O. F., and Bernhard, A., Further investigations of disturbances of blood sugar equilibrium in their relation to neoplasia.....	223
Salts, The influence upon the growth of transplanted Flexner-Jobling rat carcinoma of hydrogen ions and of, in various concentrations.....	
Sarcoma, Influence of the lymphocyte on the peritoneal implantation of, in mice.....	41
Sarcomatous tumors, Inoculation of, into negro fowls, with special reference to the significance of chromatophores.....	31
Sensitization to heat by radiation, Further experiments on (summary).....	193
Simmons, Channing C., and Greenough, Robert B., End-result reports of cancer operations (summary).....	187
Sivertsen, Ivar, and Dahlstrom, A. W., The relation of muscular activity to carcinoma. A preliminary report.....	365
Slye, Maud, The influence of heredity in determining tumor metastases. Studies in the incidence and inheritability of spontaneous tumors in mice. Sixteenth report.....	139

Slye, Maud, The influence of heredity in determining tumor metastasis. Studies in the incidence and inheritability of spontaneous tumors in mice. Fifteenth report (summary).....	182
—, Holmes, Harriet F., and Wells, H. Gideon. Primary spontaneous squamous cell carcinomas in mice. Studies on the incidence and inheritability of spontaneous tumors in mice. Fifteenth communication..	57
—, Holmes, Harriet F., and Wells, H. Gideon, Primary spontaneous tumors in the kidney and adrenal of mice. Studies on the incidence and inheritability of spontaneous tumors in mice. Seventeenth communication	305
—, Holmes, Harriet F., and Wells, H. Gideon. Primary spontaneous tumors of the ovary in mice. Studies in the incidence and inheritability of spontaneous tumors in mice. Fourteenth report (summary).....	92
—, Holmes, Harriet F., and Wells, H. Gideon, The occurrence of squamous cell carcinoma in mice (summary).....	180
Smith, George Gilbert, Radium in cancer of the bladder (summary).....	190
Squamous cell carcinoma in mice, The occurrence of (summary).....	180
Strong, Wendell M., Is cancer mortality increasing?.....	251
Sugiura, Kanematsu, Noyes, Helen Miller, and Falk, K. George, The influence upon the growth of transplanted Flexner-Jobling rat carcinoma of hydrogen ions and of various salts in different concentrations.	285
Sugiura, K., and Benedict, Stanley, R., The influence of certain diets upon tumor susceptibility and growth in albino rats (summary).....	100
Teratomas and their relation to age.....	261
Theis, Ruth C., The protein content of the whole blood and plasma in cancer.	127
Thyroid, Malignant tumors of the (summary).....	194
Tissue, islet, An atypical adenoma of the pancreas originating in.....	277
Tumor age and tumor incidence.....	197
—, biology, A phase of (summary).....	96
—, growth, Parabiosis and.....	121
—, growth, Pregnancy and.....	245
—, metastases, The influence of heredity in determining.....	139
—, metastasis, The influence of heredity in determining (summary).....	182
— susceptibility and growth in albino rats, The influence of certain diets upon (summary).....	100
—, transplantable, Factors underlying susceptibility to a, in mice (summary).....	106
Tumors, Experimental production of (summary).....	196
— in mice, Further investigations on the origin of.....	197
—, Malignant, of the thyroid (summary).....	194
—, Primary spontaneous, in the kidney and adrenal of mice.....	305
—, primary spontaneous, of the ovary in mice (summary).....	92
—, sarcomatous, Inoculation of, into negro fowls with special reference to the significance of chromatophores.....	31
—, spontaneous, Studies on the incidence and inheritability of, in mice.....	57, 139, 305
—, spontaneous, Studies in the incidence and inheritability of, in mice (summary).....	182

Tumors, transplantable, Effect of blood from immune animals upon	25
—, transplanted animal, The effect of combined heat and radiation upon (summary).....	101
—, transplanted spontaneous, Effect of a reduction of lymphocytes on the growth rate of, in mice.....	1
Wells, H. Gideon, Slye, Maud, and Holmes, Harriet F., Primary spontaneous squamous cell carcinomas in mice. Studies on the incidence and inheritability of spontaneous tumors in mice. Fifteenth communication.....	57
—, Slye, Maud, and Holmes, Harriet F., Primary spontaneous tumors in the kidney and adrenal of mice. Studies on the incidence and inheritability of spontaneous tumors in mice. Seventeenth communication....	305
—, Slye, Maud, and Holmes, Harriet F., Primary spontaneous tumors of the ovary in mice. Studies in the incidence and inheritability of spontaneous tumors in mice. Fourteenth report (summary).....	92
—, Slye, Maud, and Holmes, Harriet F., The occurrence of squamous-cell carcinoma in mice (summary).....	180
Wilson, Louis B., Malignant tumors of the thyroid (summary).....	194
Wood, F. C., Experimental production of tumors (summary).....	196
—, and Prime, Frederick, Further studies in radiation dosage (summary)..	177
X-ray, spectra, High frequency.....	179



194
48

